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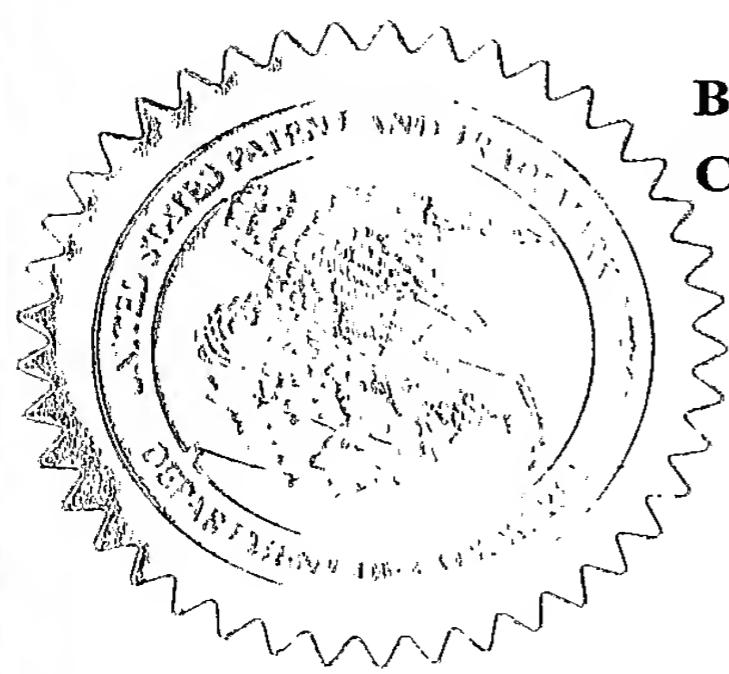
March 25, 2005

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APPLICATION NUMBER: 60/546,186

FILING DATE: February 23, 2004

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**PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Paul	FRASER	Canada			
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Inhibitors Of Islet Amyloid Polypeptide (IAPP) Fibril Formation And Uses Thereof					
Direct all correspondence to: <b>CORRESPONDENCE ADDRESS</b>					
<input checked="" type="checkbox"/> Customer Number	1059				
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<input checked="" type="checkbox"/> Firm or Individual Name	Bereskin & Parr				
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Country	Canada	Telephone	416-364-7311	Fax	416-361-1398
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages	51	<input type="checkbox"/> CD(s), Number			
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets	17	<input type="checkbox"/> Other (specify)			
<input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.					FILING FEE AMOUNT (\$)
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Respectfully submitted,  
SIGNATURE   
[Page 1 of 2] Date 02/20/04  
TYPED or PRINTED NAME Anita Nador REGISTRATION NO. 47,366  
TELEPHONE 416-957-1684 Docket Number: 2223-170

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This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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# FEE TRANSMITTAL

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Effective 10/01/2003. Patent fees are subject to annual revision.

 Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$)

80.00

## Complete if Known

Application Number	
Filing Date	
First Named Inventor	FRASER, Paul
Examiner Name	
Art Unit	
Attorney Docket No.	2223-170

## METHOD OF PAYMENT (check all that apply)

 Check  Credit card  Money Order  Other  None
 Deposit Account:

Deposit Account Number: **022095**  
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## FEE CALCULATION

## 1. BASIC FILING FEE

Large Entity	Small Entity	Fee Code (\$)	Fee Code (\$)	Fee Description	Fee Paid
1001 770	2001 385	Utility filing fee			
1002 340	2002 170	Design filing fee			
1003 530	2003 265	Plant filing fee			
1004 770	2004 385	Reissue filing fee			
1005 160	2005 80	Provisional filing fee		80.00	
<b>SUBTOTAL (1)</b>		<b>(\$)</b>	<b>80.00</b>		

## 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Extra Claims	Fee from below	Fee Paid
Independent Claims	- 20 * =	X	= 0.00
Multiple Dependent	- 3 * =	X	= 0.00

Large Entity	Small Entity	Fee Description
1202 18	2202 9	Claims in excess of 20
1201 86	2201 43	Independent claims in excess of 3
1203 290	2203 145	Multiple dependent claim, if not paid
1204 86	2204 43	** Reissue independent claims over original patent
1205 18	2205 9	** Reissue claims in excess of 20 and over original patent
<b>SUBTOTAL (2)</b>		<b>(\$)</b> 0.00

\*or number previously paid, if greater; For Reissues, see above

3. ADDITIONAL FEES	Fee Description	Fee Paid
Large Entity	Small Entity	
Fee Code (\$)	Fee Code (\$)	
1051 130	2051 65	Surcharge - late filing fee or oath
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet
1053 130	1053 130	Non-English specification
1812 2,520	1812 2,520	For filing a request for ex parte reexamination
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action
1251 110	2251 55	Extension for reply within first month
1252 420	2252 210	Extension for reply within second month
1253 950	2253 475	Extension for reply within third month
1254 1,480	2254 740	Extension for reply within fourth month
1255 2,010	2255 1,005	Extension for reply within fifth month
1401 330	2401 165	Notice of Appeal
1402 330	2402 165	Filing a brief in support of an appeal
1403 290	2403 145	Request for oral hearing
1451 1,510	1451 1,510	Petition to institute a public use proceeding
1452 110	2452 55	Petition to revive - unavoidable
1453 1,330	2453 665	Petition to revive - unintentional
1501 1,330	2501 665	Utility issue fee (or reissue)
1502 480	2502 240	Design issue fee
1503 640	2503 320	Plant issue fee
1460 130	1460 130	Petitions to the Commissioner
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)
1806 180	1806 180	Submission of Information Disclosure Stmt
8021 40	8021 40	Recording each patent assignment per property (times number of properties)
1809 770	2809 385	Filing a submission after final rejection (37 CFR 1.129(a))
1810 770	2810 385	For each additional invention to be examined (37 CFR 1.129(b))
1801 770	2801 385	Request for Continued Examination (RCE)
1802 900	1802 900	Request for expedited examination of a design application

Other fee (specify) \_\_\_\_\_

\*Reduced by Basic Filing Fee Paid

**SUBTOTAL (3)****(\$)** 0.00

## SUBMITTED BY

Name (Print/Type)	Anita Nador	Registration No. (Attorney/Agent)	47,366	Telephone (416) 364-7311	(Complete if applicable)
Signature				Date	February 20, 2004

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February 20, 2004

Anita Nador B.A. (Molec. Biophys./Biochem), LL.B.  
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Your Reference:  
Our Reference: 2223-170

The Commissioner of Patents  
& Trademarks  
Washington, D.C. 20231  
U.S.A.

Dear Commissioner:

**Re: New US Provisional Patent Application**  
**Title: Inhibitors Of Islet Amyloid Polypeptide (IAPP) Fibril Formation And**  
**Uses Thereof**  
**Inventors: FRASER, Paul**

Enclosed herewith please find the following documents regarding the above New US Provisional Patent Application that is being filed today:

- 1) Provisional Patent Application Cover Sheet;
- 2) Application Data Sheet;
- 3) Fee Transmittal; and
- 4) Provisional Patent Application.

Respectfully submitted,

**FRASER, Paul**



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B&P File No. 2223-170/AN

**BERESKIN & PARR**

**UNITED STATES**

**PROVISIONAL**

**Title: Inhibitors Of Islet Amyloid Polypeptide (IAPP) Fibril Formation And**  
**Uses Thereof**  
**Inventor: Paul Fraser**

**TITLE: Inhibitors Of Islet Amyloid Polypeptide (IAPP) Fibril Formation And Uses Thereof**

**FIELD OF THE INVENTION**

5        The invention relates to new antifibrillogenic agents, a composition containing same and a method of using these new antifibrillogenic agents. In one aspect the agents can be used to inhibit amyloid fibril formation. In another aspect they can be used as cytoprotectants. Screening methods, methods of identifying modulators of amyloid fibril formation and peptide  
10      mimetics are also encompassed within the field of the invention.

**BACKGROUND OF THE INVENTION**

15      Amyloidosis refers to a pathological condition characterized by the presence of amyloid fibers. Amyloid is a generic term referring to a group of diverse but specific protein deposits (intracellular and/or extracellular), which are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (e.g., Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share  
20      common ultrastructural features and common x-ray diffraction and infrared spectra.

25      Amyloid-related diseases can either be restricted to one organ or spread to several organs. The first instance is referred to as "localized amyloidosis" while the second is referred to as "systemic amyloidosis".

30      Some amyloidotic diseases can be idiopathic, but most of these diseases appear as a complication of a previously existing disorder. For example, primary amyloidosis can appear without any other pathology or can follow plasma cell dyscrasia or multiple myeloma. Secondary amyloidosis is usually seen associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis). A familial form of secondary amyloidosis is also seen in Familial Mediterranean Fever (FMF). This familial type of amyloidosis, as one of the other types of familial amyloidosis, is genetically inherited and is found in specific population groups. In these two

types of amyloidosis, deposits are found in several organs and are thus considered systemic amyloid diseases. Another type of systemic amyloidosis is found in long-term hemodialysis patients. In each of these cases, a different amyloidogenic protein is involved in amyloid deposition.

5 "Localized amyloidoses" are those that tend to involve a single organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-  
10 resistant form of a prion protein (referred to as AScr or PrP-27) in the central nervous system. Similarly, Alzheimer's disease (AD), another neurodegenerative disorder, is characterized by neuritic plaques and neurofibrillary tangles. In this case, the plaque and blood vessel amyloid is formed by the deposition of fibrillar A $\beta$  amyloid protein. Other diseases such  
15 as adult-onset diabetes (type 2 diabetes) are characterized by the localized accumulation of amyloid in the pancreas. Amyloid deposits are present in pancreatic islets of up to 96% of patients with Non-Insulin Dependent Diabetes (NIDDM, i.e. Type 2 diabetes) at post-mortem. These fibrillar accumulations result from the aggregation of the islet amyloid polypeptide  
20 (IAPP), also known as amylin.

Once these amyloids have formed, there is no known, widely accepted therapy or treatment which significantly dissolves the deposits *in situ*.

Each amyloidogenic protein has the ability to organize into  $\beta$ -sheets and to form insoluble fibrils that get deposited extracellularly or intracellularly.  
25 Each amyloidogenic protein, although different in amino acid sequence, has the same property of forming fibrils and binding to other elements such as proteoglycan, amyloid P and complement component. Moreover, each amyloidogenic protein has amino acid sequences which, although different, will show similarities such as regions with the ability to bind to the  
30 glycosaminoglycan (GAG) portion of proteoglycan (referred to as the GAG binding site) as well as other regions which will promote  $\beta$ -sheet formation.

This suggests that amyloid fibrils are formed by a similar protein

misfolding pathway and therefore therapeutic interventions to control their folding may be beneficial for all amyloid proteins. The associated proteins are the amyloid- $\beta$  (A $\beta$ ) protein in AD and the islet amyloid polypeptide (IAPP) in type 2 diabetes. In both AD and type 2 diabetes, amyloid plays a key role  
5 which suggests that prevention of plaque formation will have significant therapeutic benefits.

In specific cases, amyloidotic fibrils, once deposited, can become toxic to the surrounding cells. For example, the A $\beta$ fibrils organized as senile plaques, have been shown to be associated with dead neuronal cells and  
10 microgliosis in patients with Alzheimer's disease. When tested *in vitro*, A $\beta$  peptide was shown to be capable of triggering an activation process of microglia (brain macrophages), which would explain the presence of microgliosis and brain inflammation found in the brain of patients with Alzheimer's disease.

15 In another type of amyloidosis seen in patients with type 2 diabetes, and in patients with type 1 diabetes post-transplantation, the amyloidogenic protein, IAPP, has been shown to induce  $\beta$ -islet cell toxicity *in vitro*. Hence, appearance of IAPP fibrils in the pancreas of type 2 or type 1 diabetic patients could contribute to the loss of the  $\beta$ -islet cells (Langerhans) and organ  
20 dysfunction.

#### Islet Amyloid Polypeptide And Diabetes

Amyloid deposits are present in pancreatic islets of up to 96% of patients with Non-Insulin Dependent Diabetes (NIDDM, i.e, type 2) at post-  
25 mortem. These fibrillar accumulations result from the aggregation of the islet amyloid polypeptide (IAPP) or amylin, which is a 37 amino acid peptide, derived from a larger precursor peptide, pro-IAPP. IAPP co-localizes and is co-secreted with insulin in response to  $\beta$ -cell secretagogues. This pathological feature is not associated with insulin-dependent diabetes (type 1 diabetes)  
30 and is a unifying characteristic for the heterogeneous clinical phenotypes diagnosed as NIDDM (type 2 diabetes). The causal factors for islet amyloidosis and its role in the disease process have yet to be determined.

However, longitudinal studies in cats and immunocytochemical investigations in monkeys have shown that a progressive increase in islet amyloid is associated with a dramatic decrease in the population of insulin-secreting  $\beta$ -cells and increased severity of the disease. More recently, transgenic

5 approaches have strengthened the relationship of IAPP plaque formation and  $\beta$ -cell dysfunction, which indicates that amyloid deposition is a principal factor in type 2 diabetes. Amyloid accumulations are also likely to be underestimated and much more extensive since the low resolution histological dyes currently used are unable to detect anything other than large deposits.

10 Islet hyalinosis (amyloid deposition) was first described over a century ago as the presence of fibrous protein aggregates in the pancreas of patients with severe hyperglycemia (Opie, E.L., *J Exp. Med.* 5: 397-428, 1990). Today, islet amyloid, composed predominantly of islet amyloid polypeptide (IAPP), or amylin, is a characteristic histopathological marker in over 90% of all cases of

15 type 2 diabetes.

The mature IAPP molecule is a 37 amino acid residue peptide synthesized in the pancreas, and is co-localized with insulin in  $\beta$ -cell dense core secretory granules. Since IAPP is co-secreted with insulin, it has been suggested that IAPP plays a role in regulating blood glucose by controlling

20 insulin secretion. The presence of soluble IAPP in the plasma itself is normally not problematic. In patients with type 2 diabetes, however, the accumulation of pancreatic IAPP leads to a buildup of IAPP-amyloid as insoluble fibrous deposits which eventually replace the insulin-producing  $\beta$  cells of the islet resulting in  $\beta$  cell depletion and failure (Westermark, P., Grimelius, L., *Acta*

25 *Path. Microbial Scand, sect. A.* 81: 291-300, 1973; de Koning, E.J.P. et al., *Diabetologia* 36: 378-384, 1993; and Lorenzo, A. et al., *Nature* 368: 756-760, 1994). Amyloid lesions precede hyperglycemia suggesting that IAPP deposition is a principal cause of islet dysfunction (de Koning, E.J.P., Bodkin, N.L., Hansen, B.C. and Clark, A. (1993) *Diabetologia* 36:378-384). Further,

30 accumulation of IAPP results in a significantly decreased  $\beta$  cell mass in both human and non-human primates (Clark, A., Wells, C.A., Buley, I.D., Cruickshank, J.D., Vangegan, R.I., et al. (1988) *Diabetes Res.* 9:151-159).

Cumulatively, these observations suggest a close link between islet amyloid and the progression of type 2 diabetes.

Genetic and biochemical investigations have implicated amyloid as a primary and causative agent, for example, in Alzheimer's disease. However, 5 it has become apparent that the relationship between IAPP and diabetes is part of a more complex cascade involving several interconnected factors. Research to date has indicated that type 2 diabetes is initiated by other factors such as peripheral insulin resistance or obesity factors. These factors result in a heightened metabolism of  $\beta$  cells and a subsequent increase in 10 insulin secretion. This chain of events is predicted to result in an abnormally high and localized concentration of the co-secreted IAPP that culminates in extracellular and vascular amyloid deposition. These fibrillar accumulations, either through direct toxicity and/or by impeding the diffusion of nutrient, contribute to islet dysfunction and ultimately the cellular pathology of type 2 15 diabetes.

It has been suggested that differing levels of glycosylation may lead to a pool of peptides that are more apt to be involved in aggregation. Other studies have suggested that in type 2 diabetes, incomplete enzymatic processing of IAPP from its precursor pro-IAPP by the prohormone convertase PC2 may 20 provide a level of aggregatable peptide needed for the "seeding" of amyloid fibrils. Still other studies have examined the properties contained in the amino acid sequence of human IAPP that make it prone to aggregation as compared to rodent IAPP which does not form typical amyloid fibrils (Johnson, KH. et al., *N. Engl J. Med* 321: 513-518, 1989; and Moriarty, DF., Raleigh, DP. 25 *Biochemistry* 38: 1811-1818, 1999).

IAPP amyloid has many features in common with cerebral amyloid formed in Alzheimer's disease from the amyloid- $\beta$  (A $\beta$ ) peptide. Both amyloid diseases are progressive and age-related and associated with irreversible deterioration in cellular function. Neither pathological conditions require 30 synthesis of a mutated form of the peptide and both component peptides are derived from a larger precursor and form morphologically similar amyloid fibrils.

Islet Amyloid Polypeptide And Cell Death

Diseases caused by the death or malfunctioning of a particular type or types of cells can be treated by transplanting into the patient healthy cells of the relevant type of cell. This approach has been used for type I diabetic

5 patients. Often these cells are cultured *in vitro* prior to transplantation to increase their numbers, to allow them to recover after the isolation procedure or to reduce their immunogenicity. However, in many instances the transplants are unsuccessful, due to the death of the transplanted cells. One reason for this poor success rate may be IAPP, which can form fibrils and  
10 become toxic to the cells *in vitro*. In addition, IAPP fibrils are likely to continue to grow after the cells are transplanted and cause death or dysfunction of the cells. This may occur even when the cells are from a healthy donor and when the patient receiving the transplant does not have a disease that is characterized by the presence of fibrils.

15 Islet Amyloid Polypeptide

As stated above, the mature IAPP molecule is a 37 amino acid residue peptide synthesized in the pancreas (Human IAPP is SEQ. ID. NO. 1). IAPP contains three principal domains that contribute to fibril formation (Figure 1; SEQ. ID. NOS. 3, 4, and 5). These domains have been identified by looking at  
20 different peptide fragments and also through the effects of the proline mutations in the rodent sequence (SEQ. ID. NO. 2), which does not form amyloid fibrils. The initial N-terminal domain, disulfide bridge (residues 2 and 7) is not critical to amyloid fibril formation. The inventors previously demonstrated that small fragments of the IAPP displayed an inhibitory activity  
25 when combined with full-length A $\beta$  1-42 or 1-40 (Fraser, WO 02/24727, 2001). It was shown that these fragments were capable of interacting with A $\beta$  and preventing aggregation by disrupting the peptide-peptide packing within the extending amyloid fibril. This would then effectively 'cap-off' the polymerization necessary for amyloid assembly. Other approaches have  
30 been used to inhibit IAPP fibril formation Kapurniotu et al., US 6,359,112, 1998, described peptides SNNFGAILSS (hIAPP, 20-29, SEQ. ID. NO. 4), GSNKGAIIGL ( $\beta$ -IAPP, 25-34, SEQ. ID. NO. 36) and HVAAGAVVGG (PrP,

110-119) (SEQ. ID. NO. 37) for inhibiting and analyzing amyloid formation. Kapurniotu et. al. further described peptides of generally between 3-15 amino acids and containing at least the active peptide sequence GA. Cooper et al., European Patent Application No. 0 289 287 disclose various hepta- and hexapeptides of IAPP, including ANFLVH and NFLVHS, for the use in diagnosing diabetes mellitus.

5 However, neither Fraser nor Kapurniotu et al., nor Cooper et al. described the minimal inhibitory domain, which would allow for small molecule mimetics. Smaller molecules are also much easier to deal with in terms of  
10 bioavailability and attenuating metabolism that is a common failing of peptide-based approaches. Further, smaller molecules make it more feasible to use a combinatorial approach to optimization of activity.

15 There is a need for the identification of small peptides that can modulate IAPP activity and that can be used in the treatment, screening and drug development of IAPP associated conditions or amyloid related disorders.

#### **SUMMARY OF THE INVENTION**

The invention relates to, *inter alia*, *in vitro* and *in vivo* inhibitors of amyloid fibril formation. These inhibitors are, e.g., peptides which are capable  
20 of controlling IAPP aggregation and amyloid formation. This property may be used advantageously in other embodiments of the invention as disclosed herein.

In one embodiment, the invention provides peptides that are truncated (penta-, tetra-, or tri -) peptides of the hexapeptides disclosed in WO  
25 02/24727, particularly, ANFLVH (SEQ. ID. NO. 11), NFLVHS (SEQ. ID. NO. 12), SNNFGA (SEQ. ID. NO. 15), and GAILSS (SEQ. ID. NO. 19), or isomers, retro or retro-inverso isomers, peptidomimetics or salts thereof, that are antifibrillrogenic agents. In one embodiment the peptides are cytoprotectants.

In another embodiment, the peptides are truncated peptides of the  
30 hexapeptides ANFLVH (SEQ ID NO. 11), or isomers, retro or retro-inverso isomers, peptidomimetics or salts thereof. In one embodiment, the peptides are ANFLV(SEQ ID NO. 22), ANFL (SEQ. ID No. 23), ANF (SEQ ID NO. 24)

or NFL (SEQ. ID.NO. 33). In another embodiment the peptides are ANFLV(SEQ ID NO. 22), ANF (SEQ ID NO. 24) or NFL (SEQ. ID.NO. 33). In yet another embodiment the peptides are ANFLV(SEQ ID NO. 22) or ANF (SEQ ID NO. 24).

5 The agents may also be all-[D] isomers, all-[L] isomers, or a mixture of [L] and [D] isomers of the peptide.

In one embodiment, the antifibrillogenic agent is a tripeptide having the formula selected from the group consisting of:

- (I) ANX (SEQ. ID. NO. 28)
- 10 (II) AXF (SEQ. ID. NO. 29)
- (III) XNF (SEQ. ID. NO. 30),

where X is any amino acid. In one embodiment X is any amino acid except for cysteine. In another embodiment, X is glycine (G).

15 In another embodiment, the antifibrillogenic agent is a tripeptide selected from the group consisting of ANF (SEQ ID NO. 24), GNF (SEQ. ID. NO. 25), AGF (SEQ. ID. NO. 26), ANG (SEQ. ID. NO. 27), an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof. In another embodiment the tripeptide is selected from the group consisting of ANF (SEQ ID NO. 24), GNF (SEQ. ID. NO. 25), AGF (SEQ. ID. NO. 26), an 20 isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.

25 These antifibrillogenic agents may advantageously be used in the treatment of, e.g., cultured pancreatic islet cells *in vitro* prior to transplantation, for the treatment of type I diabetes patients, e.g., post-transplantation, to prevent or inhibit fibril formation in the transplanted cells.

The antifibrillogenic agents and methods of the invention may advantageously be used in preventing or delaying the progression of, notably, diabetes (type 1 or type 2), and as inhibitors of fibril formation for controlling folding or deposition of amyloid proteins.

30 Another embodiment of the invention relates to peptides; or isomers, retro or retro-inverso isomers, peptidomimetics, or salts thereof, for inhibiting amyloidosis and/or for cytoprotection. Such peptides may be formed by

truncation of a hexapeptide ANFLVH (SEQ ID NO. 11) or may be tripeptide ANF (SEQ ID NO. 24), GNF (SEQ. ID. NO. 25), or AGF (SEQ. ID. NO. 26. In another embodiment the tripeptide is ANX (SEQ. ID. NO. 28), AXF (SEQ. ID. NO. 29), or XNF (SEQ. ID. NO. 30), where X is any amino acid. In one 5 embodiment, X is not cysteine.

The invention also provides tripeptides for inhibiting amyloidosis and/or for cytoprotection, where the tripeptide binds to the sequence ANFLVH (SEQ ID NO. 11). Upon binding to the sequence, fibril formation and amyloidosis are prevented. The tripeptide may desirably be ANF (SEQ ID NO 24), ANX 10 (SEQ. ID. NO. 28), AXF (SEQ. ID. NO. 29), or XNF (SEQ. ID. NO. 30), where X is any amino acid. In one embodiment X is glycine.

The invention also relates to labeled conjugates for *in vivo* imaging of amyloid deposits featuring a conjugate of formula I:



15 where z is 0 or 1;  $A_t$  is an antifibrillogenic agent as defined above;  $A_{\text{Link}}$  is a linker moiety; and  $A_{\text{lab}}$  is a labeling moiety that allows for said *in vivo* imaging. Desirably,  $A_{\text{lab}}$  is a radiolabeling moiety, and is more preferably  $^{99m}\text{Tc}$ ,  $^{99}\text{Tc}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ,  $^{97}\text{Ru}$ ,  $^{119}\text{Pd}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{111}\text{In}$ ,  $^{113m}\text{In}$ ,  $^{153}\text{Gd}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{166}\text{Ho}$ ,  $^{198}\text{Au}$ ,  $^{90}\text{Sr}$ ,  $^{89}\text{Sr}$ ,  $^{115}\text{Rh}$ ,  $^{201}\text{Tl}$ ,  $^{51}\text{Cr}$ ,  $^{67}\text{Ga}$ ,  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$  and  $^{18}\text{F}$ . The 20 labeled conjugate may also be formulated in a composition for *in vivo* imaging of amyloid deposits. Such composition may comprise a therapeutically effective amount of a labeled conjugate as defined above in association with a pharmaceutically acceptable carrier.

The invention also includes compositions for the treatment of 25 amyloidosis disorders in a patient, including a therapeutically effective amount of an antifibrillogenic agent as defined above with a pharmaceutically acceptable carrier; and methods for the treatment of amyloidosis disorders in a patient, wherein a therapeutically effective amount of the antifibrillogenic agent is administered to a patient in need of such treatment. In an 30 embodiment, the compositions of the invention may be administered in conjunction with insulin, or in conjunction with sulfonylurea and glucose sensitizers, e.g., in a treatment for diabetes.

Processes for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits or of evoking endogenous amyloid deposition once transplanted, are also disclosed. The processes include contacting such cells *in vitro* with the antifibrillogenic agent.

5 The antifibrillogenic agent causes a breakdown of amyloid deposits (the deposits having been formed by the cells prior to coming in contact with the antifibrillogenic agent). In order to optimize the survival of cells, the cells may desirably be cultured in the presence of the antifibrillogenic agent.

The invention further includes methods for treating type 1 diabetes patients post-transplantation, wherein an antifibrillogenic agent of the invention is administered to a type 1 diabetes patient, so that amyloid deposit formation and amyloidosis is inhibited, prevented and/or reduced.

10 The use of antifibrillogenic agents, compositions containing same or compounds as described above for the various methods described above, or 15 for manufacturing a medicament or a composition for use in the various methods described above, are also disclosed.

The invention also includes methods for determining an optimized peptide for inhibition of amyloidogenesis by systematic substitution of each residue of an original tripeptide of the invention. An optimized tripeptide is one 20 where inhibition is greater than the original tripeptide. Such tripeptides can be chosen from the group consisting of ANF (SEQ ID NO. 6), GNF (SEQ. ID. NO. 25), AGF (SEQ. ID. NO. 26), and NFL (SEQ. ID. NO.33). Optimized tripeptides are also encompassed within the scope of the invention. Systematic substitution of a tripeptide will result in 57 different derivatives that 25 can be tested for inhibition and compared to inhibition of the original tripeptide. The invention also includes the optimized peptide determined by this method.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, 30 however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and

scope of the invention will become apparent to those skilled in the art from this detailed description.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

5 The invention will now be described in relation to the drawings in which:

10 Figure 1 is a comparison of the human (SEQ. ID. NO. 1) and mouse (SEQ. ID. NO. 2) IAPP sequence. The figure illustrates the primary and amyloid forming domains of human IAPP and the  $\beta$ - sheet breaking proline substitutions of the mouse peptide.

15 Figure 2 shows the primary sequence of human IAPP (SEQ. ID. NO. 1) and the original series of peptides examined as described in Fraser, WO 02/24727, 2001. [SEQ. ID. NOS. 6 - 22]

20 Figure 3 shows the peptide fragments that were examined to determine minimal IAPP binding and amyloid inhibition.

25 Figures 4 A – H are graphs illustrating circular dichroism (CD) data for the truncated tripeptide – ANF (SEQ. ID. NO. 24) (4A and 4B) - indicating the transition from random coil to beta-sheet. The ANF inhibited this conformational change when combined with IAPP at a molar ratio of 1:10.

30 Modifications of this initial inhibitory sequence to generate the peptides – GNF (SEQ. ID. NO. 25) (4C and 4D), AGF (SEQ. ID. NO. 26) (4E and 4F) and NFL (SEQ. ID. NO. 33) (4G and 4H) - resulted in similar but more potent activities at significantly lower molar ratios.

35 Figure 5 A-F are electron micrographs from negatively stained preparations of the full length IAPP showing the dense network of amyloid fibrils. The inhibitory peptide, ANF(SEQ. ID. NO. 24), reduces the relative levels of fibrils and altered the morphology of the aggregates which were formed. Data for substituted tripeptides GNF, AGF, and ANG (SEQ. ID. NOS. 25 -27) are also shown. (A) Control, (B) ANF(SEQ. ID. NO. 24), (C) GNF(SEQ. ID. NO. 25), (D) AGF(SEQ. ID. NO. 26), (E) ANG(SEQ. ID. NO. 27) and (F) NFL (SEQ. ID. NO. 33).

Figure 6 is a graph illustrating the results of the toxicity assay for ANF and Related Peptides (ANFLVH (SEQ. ID. NO. 11), ANFLV (SEQ. ID. NO. 22), and ANFL (SEQ. ID. NO. 23)): rat insulinoma (RIN) cells were exposed to exogenous IAPP with and without peptide inhibitors at a molar ratio of 1:20 [IAPP:inhibitor].

5 Figure 7 is a graph illustrating the results of the toxicity assay for ANF and Related Peptides (ANFLVH (SEQ. ID. NO. 11), GNF (SEQ. ID. NO. 25), AGF (SEQ. ID. NO. 26) and ANG (SEQ. ID. NO. 27)): rat insulinoma (RIN) cells were exposed to exogenous IAPP with and without peptide inhibitors at a 10 molar ratio of 1:20 [IAPP:inhibitor].

10 Figure 8 is a schematic diagram of the human IAPP construct used to generate transgenic mice (pNASSMAR.RIP.hIAPP).

Figure 9 is an ELISA illustrating IAPP expression in wild type (WT) and transgenic (Tg+) mice under high fat and normal diet conditions.

15 Figure 10 is bar graph illustrating the amount of secreted IAPP by cultured islets that were isolated from IAPP transgenic (IAPP TG) and non-transgenic (nonTG) mice on control or high fat diets in the presence (+) and absence (-) of glucose.

20 Figure 11 illustrates the results of a cell viability assay in IAPP transgenic and non-transgenic mice at high and low glucose concentrations.

Figures 12 A and B are electron microscope images of cultured islet cells.

#### DETAILED DESCRIPTION OF THE INVENTION

25 For the purpose of the present disclosure, the following terms are defined below.

The term "peptidomimetic" includes non-peptide compounds which mimic the structural or the functional properties of a peptide.

30 The term "amyloid related disorders" includes diseases associated with the accumulation of amyloid which can either be restricted to one organ, "localized amyloidosis", or spread to several organs, "systemic amyloidosis". Secondary amyloidosis may be associated with chronic infection (such as

tuberculosis) or chronic inflammation (such as rheumatoid arthritis), including a familial form of secondary amyloidosis which is also seen in Familial Mediterranean Fever (FMF) and another type of systemic amyloidosis found in long-term hemodialysis patients. Localized forms of amyloidosis include,

5 without limitation, diabetes type 2 and any related disorders thereof, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease, Alzheimer's disease, Cerebral Amyloid Angiopathy, and prion protein related disorders. This term also includes AA amyloidoses, AL amyloidoses, hereditary systemic amyloidoses, senile

10 systemic amyloidosis, cerebral amyloidosis, dialysis-related amyloidosis, and hormone-derived amyloidoses.

"Retro isomer" includes molecules, e.g., peptides, having a reversal of the direction of the peptide backbone.

"Peptides" include isomers thereof; retro or retro-inverso isomers thereof; peptidomimetics thereof; all-[D] isomers thereof; all-[L] isomers thereof; a mixture of [L] and [D] isomers thereof; or salts thereof.

"Inverso isomer" includes molecules, e.g., peptides, having an inversion of the amino acid chirality used to make the peptide.

"Retro-inverso isomer" includes molecules, e.g., peptides, having a reversal of both the peptide backbone direction and the amino acid chirality.

"Antifibrillogenic activity" includes the ability to block or prevent an amyloidogenic protein from forming fibrils, preferably by preventing it from adopting its  $\beta$ -pleated conformation, by disrupting protofilament interactions, and/or by interfering with the side chain interactions within the folded peptide,

25 which are believed to be necessary for aggregation and fibril formation.

The term "cytoprotection" or "cytoprotective activity" includes molecules, e.g., peptides, having the ability to protect cells from amyloid-induced toxicity.

The terms "antifibrillogenic agent" and "inhibitor of fibril formation" are used herein interchangeably.

30 The present invention provides new antifibrillogenic agents or inhibitors of fibril formation for controlling folding or deposition of amyloid proteins. The present invention also provides methods to prevent or delay the progression

of diabetes and other amyloidosis disorders. The present invention further provides small peptides having inhibitory properties, and to provide agents capable of controlling IAPP aggregation and amyloid formation.

Antifibrillogenic agents of the invention for inhibiting amyloidosis and/or for cytoprotection are provided, including peptides made from truncating hexapeptides such as ANFLVH (SEQ ID NO. 11), NFLVHS (SEQ ID NO. 12), SNNFGA (SEQ ID NO. 15) and GAILSS (SEQ ID NO. 19). In one embodiment, hexapeptides are truncated to ANFLV (SEQ ID NO. 22), ANFL, or ANF (SEQ ID NO. 24) and tripeptides such as ANF (SEQ ID NO. 24), NFL (SEQ ID NO. 33), FLV (SEQ ID NO. 35), LVH (SEQ ID NO. 34). In another embodiment, the peptides are ANFLV (SEQ ID NO. 22), ANF (SEQ ID NO. 24) or NFL (SEQ ID NO. 33)

In an embodiment of the invention, peptides for inhibiting amyloidosis and/or for cytoprotection are provided, wherein the peptide has a sequence selected from truncated hexapeptide ANFLVH (SEQ ID NO. 1). In one embodiment the hexapeptide is truncated to ANFLV (SEQ ID NO. 22), ANFL (SEQ. ID. NO. 23) or ANF (SEQ ID NO. 24) and to tripeptides such as ANF (SEQ ID NO. 24), NFL (SEQ ID NO. 33), FLV (SEQ ID NO. 35), LVH (SEQ ID NO. 34). In another embodiment, the peptides are ANFLV (SEQ ID NO. 22), ANF (SEQ ID NO. 24) or NFL (SEQ ID NO. 33)

Another embodiment of the invention relates to peptides for inhibiting amyloidosis and/or for cytoprotection, where the peptide binds to a sequence selected from ANFLVH (SEQ ID NO. 11), NFLVHS (SEQ ID NO. 12), SNNFGA (SEQ ID NO. 15) and GAILSS (SEQ ID NO. 19), and upon peptide binding to the sequence, prevents fibril formation and amyloidosis.

In a further embodiment of the invention, the invention relates to methods of determining optimized tripeptides for inhibiting amyloidosis and/or for cytoprotection, comprising

- (a) choosing an original peptide from the group consisting of ANF (SEQ ID NO. 24), NFL (SEQ ID NO. 33), FLV (SEQ ID NO. 35), LVH (SEQ ID NO. 34);
- (b) systematically substituting at each residue a different amino acid;
- (c) testing the ability for each derivative to inhibit amyloid fibril formation,

(d) comparing the inhibition of each derivative with the inhibition of the original peptide,

wherein an increase in inhibition of the derivative over the original peptide indicates an optimized peptide.

5 For example, if the original peptide consisted of the 123 amino acid sequence, test derivatives would be X23, 1X3, and 12X, wherein X is selected from the group consisting of amino acids Gly, Ala, Val, Leu, Ile, Ser, Thr, met, Asp, Asn, Glu, Gln, Arg, Lys, His, Phe, Tyr, Trp and Pro. In one embodiment, the original peptide is ANF (SEQ. ID. NO. 24) and the optimized peptides are  
10 either XNF(SEQ. ID. NO. 30), AXF(SEQ. ID. NO. 29) or ANX(SEQ. ID. NO. 28). This systematic substitution generates 57 different derivatives for each original tripeptide. Inhibitory activity can be determined using at least one of the *in vitro* assay systems described below: CD, EM and cell toxicity. In a further embodiment, the invention relates to the optimized peptide determined  
15 by this method.

The antifibrillogenic agents can be formulated in a composition for inhibiting amyloidosis and/or for cytoprotection, and include a therapeutically effective amount of antifibrillogenic agents of the invention in association with a pharmaceutically acceptable carrier.

20 Another embodiment of the invention relates to compounds for inhibiting amyloidosis and/or for cytoprotection which bind with a peptide as defined above. The compounds may be, e.g., an enzyme that binds to or controls the expression of the peptide, or an antibody that binds to the peptide. Such antibody may be specific for the peptide and may be either a monoclonal or  
25 polyclonal antibody.

Agents of the invention may be used for the *ex vivo* preparation of cells, e.g., in culture, suitable for transplantation into a mammal, e.g., islet cells, which cells are capable of forming amyloid deposits, wherein in the preparation of the cells to be transplanted, the cells are contacted with the  
30 antifibrillogenic agent. The antifibrillogenic agent causes a breakdown of amyloid deposits (the deposits having been formed by the cells prior to coming in contact with the antifibrillogenic agent).

The agents of the invention may advantageously be used in treating type 1 diabetes patients post transplantation, wherein an antifibrillogenic agent is administered to a type 1 diabetes patient for inhibiting, preventing and/or reducing amyloid deposit formation and amyloidosis. The 5 antifibrillogenic agent may be administered in conjunction with insulin.

The antifibrillogenic agents may also be used for inhibiting amyloidosis and/or for cytoprotection, wherein a therapeutically effective amount of the antifibrillogenic agent is administered to a subject, such that the antifibrillogenic agent prevents or reduces amyloid deposition; The 10 antifibrillogenic agent may desirably be administered by cell therapy or gene therapy wherein the cells have been modified to produce and secrete the antifibrillogenic agent. Such cells may be modified *ex vivo* or *in vivo*.

The antifibrillogenic agents of the invention may also be used for imaging plaques, in which case the antifibrillogenic agents, e.g., peptides, are 15 amyloid targeting imaging agents of the following formula:



where  $z$  is 0 or 1;  $A_t$  is the antifibrillogenic agent of IAPP fibril formation as described herein;  $A_{\text{link}}$  is a linker moiety; and  $A_{\text{lab}}$  is a labeling moiety.

Labeling moiety  $A_{\text{lab}}$  allows the amyloid targeting imaging agent, once at the 20 target site *in vivo*, to be visualized by instrumentation such as CT, MRI, ultrasound, radioisotopic or fluorescence detection. The labeling moiety either modulates an externally applied energy or generates a detectable energy itself. The labeling moiety may be an echogenic substance in the case of an ultrasound contrast agent, a paramagnetic metal chelate in the case of an 25 MRI contrast agent, a radioactive atom (e.g., radioactive fluorine) or a chelated radioactive metal ion (e.g., In-III) in the case of a radionuclide imaging agent, a radio-opaque chelate or compound (e.g., a polyiodinated aromatic) for an x-ray contrast agent, or a fluorescent or colored dye in the case of an optical imaging contrast agent. In one embodiment labeling moiety 30  $A_{\text{lab}}$  may be a metal chelator. In an advantageous embodiment,  $A_{\text{lab}}$  is a radionuclide (either a chelate of a metal ion or a single atom) or a

paramagnetic metal ion chelate. According to one aspect of the invention, a labeled targeting molecule-chelator conjugate comprises a labeling moiety  $A_{lab}$  (e.g., a radionuclide) attached directly to amyloid-targeting moiety  $A_t$ , therefore not requiring the use of a linker moiety.

5 Preferably,  $A_{lab}$  includes a radionuclide selected from  $^{99m}Tc$ ,  $^{99}Tc$ ,  $^{64}Cu$ ,  $^{67}Cu$ ,  $^{97}Ru$ ,  $^{119}Pd$ ,  $^{186}Re$ ,  $^{188}Re$ ,  $^{111}In$ ,  $^{113m}In$ ,  $^{153}Gd$ ,  $^{90}Y$ ,  $^{153}Sm$ ,  $^{166}Ho$ ,  $^{198}Au$ ,  $^{90}Sr$ ,  $^{89}Sr$ ,  $^{115}Rh$ ,  $^{201}TI$ ,  $^{51}Cr$ ,  $^{67}Ga$ ,  $^{57}Co$ ,  $^{60}Co$ ,  $^{123}I$ ,  $^{125}I$ ,  $^{131}I$  and  $^{18}F$ .

As an imaging agent,  $A_{lab}$  preferably includes a radionuclide selected from the group consisting of Tc and Re. More preferably,  $A_{lab}$  is a metal 10 chelate of a radioactive or paramagnetic metal ion.

In both AD and type 2 diabetes, amyloid plays a key role. The antifibrillogenic agents of the invention may be peptides, peptidomimetics, antibodies, or other compounds, that interact or interfere with either or both regions of the amyloidogenic peptide that are involved in amyloid formation,

15 ATQRLANFLVHSS (SEQ. ID. NO. 38) and SSNNFGAILSSTN (SEQ. ID. NO. 39) in the case of the IAPP peptide. The antifibrillogenic agents may also be enzymes that bind to or control the expression of the amyloidogenic peptide.

When the antifibrillogenic agents are peptides, all-[D] peptides, all-[L] peptides and peptides which are a mixture of [L] and [D] isomers are included.

20 Without wishing to be limited to a particular theory or interpretation of how the invention operates, antifibrillogenic agents are believed to "interfere" with the amyloidogenic peptide by binding and disrupting the folding into the amyloidogenic  $\beta$ -sheet conformation, disrupting protofilament interactions, and/or interfering with the side chain interactions within the folded peptide, 25 which are necessary for aggregation and fibril formation.

The antifibrillogenic agents of the invention may be peptides, which can be modified or substituted analogs. Some analogs also include unnatural amino acids or modifications of N or C terminal amino acids. Unnatural amino acids include D-amino acids,  $\alpha,\alpha$ -disubstituted amino acids, N-alkyl amino acids, lactic acid, 4-hydroxyproline, gamma-carboxyglutamate,  $\epsilon$ -N,N,N-trimethyllysine,  $\epsilon$ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine,  $\Omega$ -N-methylarginine and

isoaspartic acid.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (e.g., 99 percent sequence identity). Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

The term "antibody" or "immunoglobulin" is used to include intact antibodies and binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to an antigen fragment including separate heavy chains, light chains Fab, Fab', 25 F(ab')<sub>2</sub>, Fabc, and Fv. Fragments are produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins. The term "antibody" also includes one or more immunoglobulin chains that are chemically conjugated to, or expressed as, fusion proteins with other proteins. The term "antibody" also includes bispecific antibody. A bispecific or 30 bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or

linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelný et al., *J. Immunol.* 148, 1547-1553 (1992). Specific binding between two entities means an affinity of at least  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9 M^{-1}$  or  $10^{10} M^{-1}$ . Affinities greater than  $10^8 M^{-1}$  are preferred.

5 A "pharmaceutical composition" refers to a chemical or biological composition suitable for administration to a mammalian individual. Such compositions may be specifically formulated for administration via one or more of a number of routes, including but not limited to, oral, parenteral, intravenous, intraarterial, subcutaneous, intranasal, sublingual, intraspinal, 10 intracerebroventricular, and the like. A "pharmaceutical excipient" or a "pharmaceutically acceptable excipient" is a carrier, usually a liquid, in which an active therapeutic peptide is formulated. The excipient generally does not provide any pharmacological activity to the formulation, though it may provide chemical and/or biological stability, release characteristics, and the like. 15 Exemplary formulations can be found, for example, in Remington's Pharmaceutical Sciences, 19th Ed., Gennaro, A., Ed., 1995.

Such peptides, proteins or fragments, analogs and other amyloidogenic peptides may be synthesized by solid phase peptide synthesis or recombinant expression, according to standard methods well known in the art, or can be 20 obtained from natural sources. Automatic peptide synthesizers may be used, and are commercially available from numerous manufacturers, such as Applied Biosystems (Perkin Elmer; Foster City, California), and procedures for preparing synthetic peptides are known in the art.

Antifibrillogenic agents of the invention may also be derived from the 25 peptides by substitution of one or more residues in the naturally occurring sequence. In another embodiment, the agents are peptidomimetics of the peptides. The agents may be modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acids or non-amino acid fragments, such as thienylalanine, 30 cyclohexylalanine and phenylglycine.

The antifibrillogenic agents, e.g., peptides, may be used to actively immunize a patient, so that patient after immunization will produce antibodies

that will recognize the peptide sequence against which they have been raised. Alternatively, peptide antifibrillogenic agents of the invention can be used for producing antibodies to be administered to patients for passive immunization. The antibodies administered (in the case of a passive immunization) or the 5 antibodies produced by the patients (in the case of an active immunization) will recognize a sequence on the IAPP corresponding to the sequence against which they have been raised, for inhibiting or reducing plaque formation.

As an non-limiting illustration of the utility of the invention, the following types of amyloidosis are described in more detail below.

10

#### **AA (reactive) Amyloidosis**

Generally, AA amyloidosis is a manifestation of a number of diseases that provoke a sustained acute phase response. Such diseases include chronic inflammatory disorders, chronic local or systemic microbial infections, 15 and malignant neoplasms.

AA fibrils are generally composed of 8000 dalton fragments (AA peptide or protein) formed by proteolytic cleavage of serum amyloid A protein (apoSSA), a circulating apolipoprotein which is present in HDL complexes and which is synthesized in hepatocytes in response to such cytokines as IL-1, IL-20 6 and TNF. Deposition can be widespread in the body, with a preference for parenchymal organs. The spleen is usually a deposition site, and the kidneys may also be affected. Deposition is also common in the heart and gastrointestinal tract.

AA amyloid diseases include, but are not limited to inflammatory 25 diseases, such as rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis, psoriasis, psoriatic arthropathy, Reiter's syndrome, Adult Still's disease, Behcet's syndrome, and Crohn's disease. AA deposits are also produced as a result of chronic microbial infections, such as leprosy, tuberculosis, bronchiectasis, decubitus ulcers, chronic pyelonephritis, 30 osteomyelitis, and Whipple's disease. Certain malignant neoplasms can also result in AA fibril amyloid deposits. These include such conditions as

Hodgkin's lymphoma, renal carcinoma, carcinomas of gut, lung and urogenital tract, basal cell carcinoma, and hairy cell leukemia.

### **AL Amyloidoses**

5 AL amyloid deposition is generally associated with almost any dyscrasia of the B lymphocyte lineage, ranging from malignancy of plasma cells (multiple myeloma) to benign monoclonal gammopathy. At times, the presence of amyloid deposits may be a primary indicator of the underlying dyscrasia.

10 Fibrils of AL amyloid deposits are composed of monoclonal immunoglobulin light chains or fragments thereof. More specifically, the fragments are derived from the N-terminal region of the light chain (kappa or lambda) and contain all or part of the variable (VL) domain thereof. Deposits generally occur in the mesenchymal tissues, causing peripheral and

15 autonomic neuropathy, carpal tunnel syndrome, macroglossia, restrictive cardiomyopathy, arthropathy of large joints, immune dyscrasias, myelomas, as well as occult dyscrasias. However, it should be noted that almost any tissue, particularly visceral organs such as the heart, may be involved.

### **20 Hereditary Systemic Amyloidoses**

There are many forms of hereditary systemic amyloidoses. Although they are relatively rare conditions, adult onset of symptoms and their inheritance patterns (usually autosomal dominant) lead to persistence of such disorders in the general population. Generally, the syndromes are attributable

25 to point mutations in the precursor protein leading to production of variant amyloidogenic peptides or proteins. Table 1 summarizes the fibril composition of exemplary forms of these disorders.

**Table 1**

<b><i>Fibril Peptide/Protein</i></b>	<b><i>Genetic variant</i></b>	<b><i>Clinical Syndrome</i></b>
Transthyretin and fragments (ATTR)	Met30, many others	Familial amyloid polyneuropathy (FAP), (Mainly peripheral nerves)
Transthyretin and fragments	Thr45, Ala60, Ser84,	Cardiac involvement

(ATTR)	Met111, Ile122	predominant without neuropathy
N-terminal fragment of Apolipoprotein A1 (apoAI)	Arg26	Familial amyloid polyneuropathy (FAP), (mainly peripheral nerves)
N-terminal fragment of Apolipoprotein A1 (AapoAI)	Arg26, Arg50, Arg60, others	Ostertag-type, non-neuropathic (predominantly visceral involvement)
Lysozyme (Alys)	Thr56, His67	Ostertag-type, non-neuropathic (predominantly visceral involvement)
Fibrogen $\gamma$ chain fragment	Leu554, Val526	Cranial neuropathy with lattice corneal dystrophy
Gelsolin fragment (Agel)	Asn187, Tyr187	Cranial neuropathy with lattice corneal dystrophy
Cystatin C fragment	Glu68	Hereditary cerebral hemorrhage (cerebral amyloid angiopathy)- Icelandic type
$\beta$ -amyloid protein (a $\beta$ ) derived from Amyloid Precursor Protein (APP)	Gln693	Hereditary cerebral hemorrhage (cerebral amyloid angiopathy)- Dutch type
$\beta$ -amyloid protein (a $\beta$ ) derived from Amyloid Precursor Protein (APP)	Ile717, Phe717, Gly717	Familial Alzheimer's Disease
$\beta$ -amyloid protein (a $\beta$ ) derived from Amyloid Precursor Protein (APP)	Asn670, Leu671	Familial Dementia – probably Alzheimer's Disease
Prion Protein (PrP) derived from Prp Precursor protein 51-91 insert	Leu102, Val167, Asn178, Lys200	Familial Creutzfeldt-Jakob disease; Gerstmann-Sträussler-Scheinker syndrome (hereditary spongiform encephalopathies, prion diseases)
AA derived from Serum amyloid A protein (ApoSSA)		Familial Mediterranean fever, predominant renal involvement (autosomal recessive)
AA derived from Serum amyloid A protein (ApoSSA)		Muckle-Wells syndrome, nephropathy, deafness, urticaria, limb pain
Unknown		Cardiomyopathy with persistent atrial standstill

Unknown		Cutaneous deposits (bulous, papular, pustulodermal)
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\*Data derived from Tan & Pepys, *Histopathology*, 25(5): 403-414, 1994.

The data provided in Table 1 are exemplary and are not intended to limit the scope of the invention. For example, more than 40 separate point mutations in the transthyretin gene have been described, all of which give rise 5 to clinically similar forms of familial amyloid polyneuropathy.

Transthyretin (TTR) is a 14 kilodalton protein that is also sometimes referred to as prealbumin. It is produced by the liver and choroid plexus, and it functions in transporting thyroid hormones and vitamin A. At least 50 variant forms of the protein, each characterized by a single amino acid change, are 10 responsible for various forms of familial amyloid polyneuropathy. For example, substitution of proline for leucine at position 55 results in a particularly progressive form of neuropathy; substitution of methionine for leucine at position 111 resulted in a severe cardiopathy in Danish patients. Amyloid deposits isolated from heart tissue of patients with systemic amyloidosis have 15 revealed that the deposits are composed of a heterogeneous mixture of TTR and fragments thereof, collectively referred to as ATTR, the full length sequences of which have been characterized. ATTR fibril components can be extracted from such plaques and their structure and sequence determined according to the methods known in the art.

20 Persons having point mutations in the molecule apolipoprotein AI (e.g., Gly → Arg26; Trp 4 → Arg50; Leu → 4 Arg60) exhibit a form of amyloidosis ("Östertag type") characterized by deposits of the protein apolipoprotein AI or fragments thereof (AApoAI). These patients have low levels of high density lipoprotein (HDL) and present with a peripheral neuropathy or renal failure.

25 A mutation in the alpha chain of the enzyme lysozyme (e.g., Ile → Thr56 or Asp → His57) is the basis of another form of Östertag-type non-neuropathic hereditary amyloid reported in English families. Here, fibrils of the mutant lysozyme protein (Alys) are deposited, and patients generally exhibit impaired renal function. This protein, unlike most of the fibril-forming proteins 30 described herein, is usually present in whole (unfragmented) form.

β-amyloid peptide (A<sub>β</sub>) is a 39-43 amino acid peptide derived by proteolysis from a large protein known as Beta Amyloid Precursor protein (βAPP). Mutations in βAPP result in familial forms of Alzheimer's disease, Down's syndrome and/or senile dementia, characterized by cerebral

5 deposition of plaques composed of A<sub>β</sub> fibrils and other components, which are described in further detail below. Known mutations in APP associated with Alzheimer's disease occur proximate to the cleavage sites of β or gamma-secretase, or within A<sub>β</sub>. For example, position 717 is proximate to the site of gamma-secretase cleavage of APP in its processing to A<sub>β</sub>, and positions

10 670/671 are proximate to the site of β-secretase cleavage. Mutations at any of these residues may result in Alzheimer's disease, presumably by causing an increase in the amount of the 42/43 amino acid form of A<sub>β</sub> generated from APP. The structure and sequence of A<sub>β</sub> peptides of various lengths are well known in the art. Such peptides can be made according to methods known in

15 the art (e.g., Glenner and Wong, Biochem Biophys. Res. Comm. 129: 885-890, 1984; Glenner and Wong, Biochem Biophys. Res. Comm. 122: 113 1-1135, 1984). In addition, various forms of the peptides are commercially available.

Synuclein is a synapse-associated protein that resembles an

20 alipoprotein and is abundant in neuronal cytosol and presynaptic terminals. A peptide fragment derived from alpha-synuclein, termed NAC, is also a component of amyloid plaques of Alzheimer's disease.

Gelsolin is a calcium binding protein that binds to fragments and actin filaments. Mutations at position 187 (e.g., Asp→Asn; Asp→Tyr) of the protein

25 result in a form of hereditary systemic amyloidosis, usually found in patients from Finland, as well as persons of Dutch or Japanese origin. In afflicted individuals, fibrils formed from gelsolin fragments (Agel), usually consist of amino acids 173-243 (68 kDa carboxyterminal fragment) and are deposited in blood vessels and basement membranes, resulting in corneal dystrophy and

30 cranial neuropathy which progresses to peripheral neuropathy, dystrophic skin changes and deposition in other organs.

Other mutated proteins, such as mutant alpha chain of fibrinogen (AfibA) and mutant cystatin C (Acys) also form fibrils and produce characteristic hereditary disorders. AfibA fibrils form deposits characteristic of a nonneuropathic hereditary amyloid with renal disease; Acys deposits are 5 characteristic of a hereditary cerebral amyloid angiopathy reported in Iceland. In at least some cases, patients with cerebral amyloid angiopathy (CAA) have been shown to have amyloid fibrils containing a non-mutant form of cystatin C in conjunction with beta protein.

Certain forms of prion disease are now considered to be inheritable, 10 accounting for up to 15% of cases, which were previously thought to be predominantly infectious in nature (Baldwin et al., in Research Advances in Alzheimer's Disease and Related Disorders, John Wiley and Sons, New York, 1995). In such prion disorders, patients develop plaques composed of abnormal isoforms of the normal prion protein (prPSc). A predominant mutant 15 isoform, PrPSc, also referred to as AScr, differs from the normal cellular protein in its resistance to protease degradation, insolubility after detergent extraction, deposition in secondary lysosomes, post-translational synthesis, and high  $\beta$ -pleated sheet content. Genetic linkage has been established for at least five mutations resulting in Creutzfeldt-Jacob disease (CJD), Gerstmann- 20 Sträussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) (Baldwin, *supra*). Methods for extracting fibril peptides from scrapie fibrils, determining sequences and making such peptides are known in the art. For example, one form of GSS has been linked to a PrP mutation at codon 102, while telencephalic GSS segregates with a mutation at codon 117. Mutations 25 at codons 198 and 217 result in a form of GSS in which neuritic plaques characteristic of Alzheimer's disease contain PrP instead of A $\beta$  peptide. Certain forms of familial CJD have been associated with mutations at codons 200 and 210; mutations at codons 129 and 178 have been found in both familial CJD and FFI (Baldwin, *supra*).

### **Senile Systemic Amyloidosis**

Amyloid deposition, either systemic or focal, increases with age. For example, fibrils of wild type transthyretin (TTR) are commonly found in the heart tissue of elderly individuals. These may be asymptomatic, clinically

5 silent, or may result in heart failure. Asymptomatic fibrillar focal deposits may also occur in the brain (A $\beta$ ), corpora amylacea of the prostate (A $\beta$ 2 microglobulin), joints and seminal vesicles.

### **Cerebral Amyloidosis**

10 Local deposition of amyloid is common in the brain, particularly in elderly individuals. The most frequent type of amyloid in the brain is composed primarily of A $\beta$  peptide fibrils, resulting in dementia or sporadic (non-hereditary) Alzheimer's disease. In fact, the incidence of sporadic Alzheimer's disease greatly exceeds forms shown to be hereditary. Fibril 15 peptides forming these plaques are very similar to those described above, with reference to hereditary forms of Alzheimer's disease (AD).

### **Dialysis-related Amyloidosis**

20 Plaques composed of  $\beta$ 2 micro globulin (A $\beta$ 2M) fibrils commonly develop in patients receiving long term hemodialysis or peritoneal dialysis.  $\beta$ 2 microglobulin is a 11.8 kilodalton polypeptide and is the light chain of Class I MHC antigens, which are present on all nucleated cells. Under normal circumstances, it is continuously shed from cell membranes and is normally filtered by the kidney. Failure of clearance, such as in the case of impaired 25 renal function, leads to deposition in the kidney and other sites (primarily in collagen-rich tissues of the joints). Unlike other fibril proteins, A $\beta$ 2M molecules are generally present in unfragmented form in the fibrils.

### **Hormone-derived Amyloidoses**

30 Endocrine organs may harbor amyloid deposits, particularly in aged individuals. Hormone-secreting tumors may also contain hormone-derived amyloid plaques, the fibrils of which are made up of polypeptide hormones

such as calcitonin (medullary carcinoma of the thyroid), islet amyloid polypeptide (amylin; occurring in most patients with type 2 diabetes), and atrial natriuretic peptide (isolated atrial amyloidosis). Sequences and structures of these proteins are well known in the art.

5

### **Miscellaneous Amyloidoses**

There are a variety of other forms of amyloid disease that are normally manifest as localized deposits of amyloid. In general, these diseases are probably the result of the localized production and/or lack of catabolism of 10 specific fibril precursors or a predisposition of a particular tissue (such as the joint) for fibril deposition. Examples of such idiopathic deposition include nodular AL amyloid, cutaneous amyloid, endocrine amyloid, and tumor-related amyloid.

15 The invention, in a particular embodiment, is especially useful for treatment of diabetes, e.g., amyloid-related diabetes. The following description sets this forth in more detail.

### **Type 2 Diabetes And IAPP**

#### **Primary Structure of IAPP and Fibril Formation**

20 There are three regions of human IAPP (hIAPP) that have the potential to form fibrils. In addition to the region 20-29, originally described as the amyloidogenic region (Betsholtz et al., *FEBS Lett* 251:261-264, 1989) and the report of hIAPP 30-37 forming fibrils (Nilsson & Raleigh, *J Mol Biol* 294:1375-1385, 1999), hIAPP 8-20 also forms fibrils (Fraser, WO 02/24727, 2001).

25 These findings suggest that 20-29 is not the only amyloidogenic region of the sequence. In addition, the fragment, rat IAPP 8-20, which has an arginine at position 18 but is otherwise homologous to hIAPP 8-20 formed fibrils in aqueous media.

30 While other studies have shown fragments of hIAPP to form fibrils rapidly in aqueous media, Fraser, *supra*, utilized a preparation of hIAPP free of 'seeds' as the starting material (Higham et al., *Eur J Biochem* 267:4998-5004, 2000) rather than preparations of undefined solubility. Under these

conditions, all peptide fragments were initially in random conformation when examined with CD and had no fibrillar structures present when examined by EM. This permitted examination of the effects of pH and counter ions on the change in peptide conformation from an unfolded state to the oligomerization

5 and formation of fibrils. Previous studies have used HFIP to stabilize IAPP in artificial helical conformation or used seeds to generate conformational changes (Kayed et al., *J Mol Biol* 287:781-796, 1999), which may not reflect the situation *in vivo*. The use of preformed seeds could preclude the formation of initial aggregation stages important in the *in vivo* generation of amyloid.

10 The three adjacent domains of hIAPP that have amyloidogenic potential may have a role in intermolecular binding, oligomerization and fibril formation as well as interacting to form intramolecular  $\beta$ -sheets. Fraser, *supra*, was the first report of fragments of rat IAPP (rat IAPP 8-20) forming fibrils. As the 30-37 region of rat IAPP is identical in amino acid structure to hIAPP and 15 therefore capable of fibril formation, it could be predicted that these two  $\beta$ -strands interact and that rat IAPP should form fibrils. The lack of fibril formation from rat IAPP suggests that the proline substitutions at rat IAPP 25, 28, and 29 prevent  $\beta$ -strand formation in this region of the peptide; these proline substitutions not only inhibit intermolecular  $\beta$ -sheet formation and 20 fibrils but also disrupt intramolecular structure that would lead to fibril formation.

The histidine residue at position 13 in A $\beta$  is important for fibril assembly. Mutant forms of A $\beta$  without histidine residues do not form structures larger than protofilaments. In rodents His13 of A $\beta$  is replaced with 25 an arginine residue, in a similar way to the Arg18His substitution that occurs in IAPP. This substitution is believed to contribute to the lack of A $\beta$  amyloid in rodents.

Fibril formation of hIAPP 1-37 is independent of pH although the morphology differs. Fraser, *supra*, demonstrated that counter-ions present in 30 the buffer influenced the morphology as well as the rate of fibril formation. Human IAPP 1-37 formed fibrils at similar rates in water and 11 mM sodium-acetate and on a shorter time scale in 2 mM Tris buffer. This was

accompanied by a conversion from random to  $\beta$ -sheet conformation as determined by CD analysis. Human IAPP 1-37 rapidly precipitated from 2 mM borate, citrate and phosphate buffers with a loss of CD signal. As the acetate and citrate buffers and the Tris and phosphate buffers were similar in ionic 5 strength and matched for pH, the differences in effect were attributed to the charge or shape of the buffer ions. Citrate and phosphate are more densely charged than acetate and Tris respectively.

Binding of zinc to the histidine residue in the A $\beta$  peptide has been proposed as an important factor for fibril assembly. The presence of His 18 10 was shown not to be essential as rat IAPP 8-20 also formed fibrils. However, in the presence of zinc, fragments 18-29 and 20-29 formed longer more loosely packed fibrils, suggesting that zinc is able to affect the packing of peptide fragments into protofilaments and assembly of protofilaments into fibrils independently of any interaction it may have with His 18 (Fraser, *supra*). 15 The highly charged zinc ion could interact with hydrophobic residues preventing lateral aggregation. A high concentration of zinc is present in the  $\beta$ -cell secretory granule which could influence the folding of IAPP.

#### **Secondary structure propensities of hIAPP**

20 Previous studies examining secondary structure predictions have produced various potential conformations for hIAPP (Hubbard et al., *Biochem J* 275:785- 788, 1991; Saldanha & Mahadevan, *Protein Eng* 4:539-544, 1991).

Structure predictions indicate that an alpha helix should be present at 25 the N terminus of hIAPP. However, the CD data in the Fraser patent application, *supra*, indicate that hIAPP is usually found either in a random coil state, a  $\beta$ -sheet, or precipitated from solution (Higham et al., *Febs Lett* 470:55-60, 2000). Only in the presence of helix promoting solvents (TFE, HFIP) does it exhibit alpha helical nature (Higham et al., *Febs Lett* 470:55-60, 30 2000). This suggests either that hIAPP, *in vitro*, does not retain its native structure or that hIAPP is unstructured and, under appropriate conditions, assumes a  $\beta$ -sheet structure more easily than other conformations.

Alternatively, hIAPP *in vivo* could exist as a random coil structure and circulate bound to a carrier to maintain stability. Although the secondary structures predicted by algorithms are based on known structures, they cannot predict whether a molecular conformation is kinetically accessible and

5 therefore possible to attain *in vitro* or *in vivo*.

The conformations determined separately for different domains of the peptide may not represent that existing in the intact molecule since fragmentation removes tertiary contacts and fibril formation of separate fragments may occur under conditions where the full-length sequence does

10 not form fibrils. Rat IAPP 8-20 will form fibrils but the full-length rat IAPP does not. However, despite the limitations of both secondary structure predictions and the difficulties of inference of structure from fragments these methods can be used to model peptides.

## 15 **Proposal of a Model for hIAPP Fibril Formation**

The presence of two/three  $\beta$ -strands in the hIAPP sequence suggests that a small  $\beta$ -sheet is at the core of the monomeric structure. This could be stabilized by side chain hydrogen bonding between the uncharged polar side chains of asparagine and/or glutamine residues.

20 Fibril formation of two/three  $\beta$ -strands is independent of pH and counter ions and is driven by hydrophobic interactions. In the hIAPP sequence, 11 of 37 residues are hydrophobic. Increased hydrophobicity during the initial stages of hIAPP fibril formation has been demonstrated (Kayed et al., *J Mol Biol* 287:781-796, 1999) suggesting that protofilament and fibril assembly

25 exposes hydrophobic groups. Uncharged polar residues such as glutamine, serine, asparagine and threonine participate in side chain hydrogen bonding. Griffiths et al. (*Journal of the American Chemical Society* 12:3539-354, 1995) suggested that residues 24-27 form a highly ordered antiparallel  $\beta$ -sheet structure when examined as a 20-29 fragment.

30 An amyloidogenic domain of hIAPP was identified using a series of overlapping peptide fragments, providing insight into molecular sequences important in amyloid fibril formation (Fraser, *supra*). Although the hIAPP 20-29

domain is clearly important, it is unlikely to act in isolation and other IAPP regions must contribute to formation/stabilization of the  $\beta$ -sheet conformation and the accompanying aggregation and fibril formation.

Fraser, *supra*, shows that there are at least two regions of IAPP involved in fibril formation, one  $\beta$ -pleated sheet region (IAPP 20-29) and one region of previously unknown function (IAPP 8-20). The antifibrillogenic agents of the present invention can act by interacting or interfering with either or both regions.

The fibril forming ability of hIAPP was pH insensitive, suggesting that the transition of IAPP *in vivo* from the  $\beta$ -cell secretory granule (pH 5.5) to the extracellular space (pH 7.4) does not have a significant effect on the conformation of the peptide. It is more likely that changes in the granule components or in the extracellular environment, which are unique to type 2 diabetes, allow fibril formation to occur. The  $\beta$ -cell granule contains more than 30 identified proteins and has high concentrations of both zinc and calcium. Intracellular molecular crowding could be essential for maintenance of hIAPP in its native conformation or inhibition of aggregation. Changes which promote 'seeding' of amyloidogenic fragments or conformational rearrangements of intact hIAPP 1-37 initiate the progressive deposition of secreted IAPP as amyloid deposits and destruction of insulin-secreting cells. Similarly, crowding effects in the extracellular space in the early stages of type 2 diabetes due to hypersecretion from the  $\beta$ -cells could result in increased concentration of hIAPP and aggregation leading to fibril formation.

The ultimate goal in the present invention is to control the disease process to prevent, delay or reverse the progression of Alzheimer's disease, diabetes or other amyloidosis disorders. Non-limiting examples of amyloidosis disorders are cerebral angiopathy, secondary amyloidosis, familial Mediterranean fever, Muckle-Wells syndrome, primary amyloidosis, familial amyloid polyneuropathy, hereditary cerebral hemorrhage, chronic hemodialysis-associated amyloidosis, and prion disorders such as Creutzfeld-Jacob disease and Gertsmann-Straussler-Scheinker syndrome.

In accordance with the invention, a series of IAPP-derived peptide fragments has been identified. These fragments have the ability to bind to the full-length protein and prevent normal folding and amyloid fibril formation. The activity of these inhibitors has been assessed, as detailed herein below, using

5 a series of biophysical techniques that include protein spectroscopy, fluorescence assays and electron microscopy.

Previous investigations demonstrated that small fragments of the amyloid- $\beta$  peptide displayed an inhibitory activity when combined with the full-length A $\beta$  1-42 or 1-40. It has been postulated that these fragments were

10 capable of interacting with A $\beta$  and preventing aggregation by disrupting the peptide-peptide packing within the extending amyloid fibril. This would then effectively 'cap-off' the polymerization necessary for amyloid assembly.

To determine if a similar strategy could be employed with IAPP, two series of overlapping hexapeptides derived from key  $\beta$ -sheet structural

15 domains within were previously synthesized and investigated (see Figure 2). The IAPP 20-29 domain has been extensively studied and is considered to be a critical region for fibril formation. This has been supported, for example, by the  $\beta$ -sheet breaking proline residues in rodent IAPP which prevent amyloid formation. More recently, investigations have indicated a second  $\beta$ -sheet

20 domain spanning residues 8-20. A third  $\beta$ -sheet forming sequence (residues 31-37) has been identified but this region was less amenable to fragment analysis due to virtually irreversible aggregation. By targeting the two domains contained within residues 8-29, generating soluble peptides capable

25 of binding to IAPP and disrupting amyloid packing and fibril formation might be possible.

Using *in vitro* assay systems, the activities of the peptide fragments were assessed and four (4) potent inhibitors were identified (Fraser, *supra*). Two peptides from the IAPP20-29 region displayed inhibitory activity - SNNFGA and GAILSS; and two from the 8-20 domain - ANFLVH and

30 NFLVHS. When combined with full-length IAPP1-37 at relatively low molar ratios of 1:5 and 1:1 [inhibitor:IAPP] these peptides were able to: (1) prevent the folding of human IAPP into a  $\beta$ -sheet conformation; (2) virtually eliminate

the assembly of IAPP fibrils as determined by electron microscopy; and (3) significantly attenuate the toxicity of IAPP fibrils in cell culture. Slight changes in sequence could result in an amyloid enhancing effect where the peptide fragments could independently assemble into amyloid fibrils. These initial 5 investigations have generated a number of interesting molecules which will be examined further and optimized in terms of their inhibitory properties.

Optimization of the Inhibitory Peptide Fragments

The initial investigations identified four hexapeptide fragments of IAPP 10 which were able to effectively inhibit amyloidogenesis and cell toxicity. To advance this technology, the following has been examined: (1) the minimal inhibitory sequence for each peptide; (2) the residues which confer activity within these sequences; (3) optimized activity through a process of systematic residue substitution; and (4) modeled the molecular structure of the most 15 active peptides in an effort to ultimately generate small molecule analogues.

The truncated peptides of the invention and/or optimized peptides of the invention can be formulated into pharmaceutical compositions with pharmaceutically acceptable carriers known in the art. They can be used in effective amounts (amounts to achieve the desired result) to modulate, such 20 as inhibit, amyloid fibril formation. In another embodiment the peptides can be used to treat amyloid-related disorders, such as diabetes or Alzheimer's. Alternatively, the peptides can be used in screening assays to identify suitable IAPP fibril formation modulators, diagnostics and peptide mimetics to develop 25 or design molecules that can be used in the treatment of amyloid-related disorders.

The following non-limiting examples are illustrative of the present invention:

**EXAMPLES**

30 **METHODS: Assays for Evaluating Inhibitor Activity:**

**1. Circular Dichroism (CD):** Amyloidogenic peptides and proteins undergo a conformational transition from a native to  $\beta$ -sheet conformation. This misfolding promotes protein-protein aggregation and the formation amyloid fibrils that have a similar morphology and structure suggestive of a common 5 assembly pathway. In the case of peptides such as A $\beta$  and IAPP, the non-fibrillar forms are essentially random coils that subsequently convert to a  $\beta$ -sheet under different conditions of pH and concentration.

CD provides a global view of the peptide secondary structure over an extended time course that can be used to monitor the amyloid conformational 10 transition (i.e., diagnostic absorption minima for  $\beta$ -conformation at 218 nm). IAPP is initially treated with hexafluoroisopropanol (HFIP) and trifluoroacetic acid (TFA) to ensure it is in a monomeric form. IAPP 1-37 (50  $\mu$ M) in the presence of inhibitors (50  $\mu$ M-1.0 mM) are compared to controls with spectra collected twice daily for a period of 72 hours ( $\beta$ -sheet conversion and precipitation 15 occurs at 48-72 hrs depending on conditions used). Inhibitors can be evaluated for their ability to maintain IAPP in a random or non- $\beta$  conformation. This can be determined by direct comparisons of the spectra obtained and by quantitative analyses using deconvolution algorithms (Jasco J720 spectropolarimeter contained in the inventor's laboratory equipped with 20 Spectra Manager).

**2. Negative Stain Electron Microscopy:** IAPP amyloid morphology and relative density of the fibrils can be examined by transmission EM techniques. This provides information of the morphology of the aggregates formed (e.g., 25 amorphous, fibrillar, truncated/protofilament structures, and/or abnormal lateral aggregation profiles). In addition, scanning of multiple grids and samples can be performed by an experienced EM technician who will assess the relative amount of fibrils formed in the presence of each inhibitor. This supporting evidence can be used to rank the relative activities of the 30 inhibitors. Aliquots are taken from the samples used for CD analysis and applied to pioloform coated grids, blotted dry and stained with phosphotungstic acid (pH 7.0).

**3. IAPP Amyloid Toxicity:** Amyloid fibrils cytotoxicity may be due to an apoptotic mechanism and/or mechanical disruption of the plasma membrane.

The ability of inhibitors to prevent toxicity in cells exposed to exogenous

5 amyloid is another indication of activity that is assessed. A rat insulinoma line (RIN-1056A) having  $\beta$  cell-like characteristics is exposed to 10  $\mu$ M IAPP 1-37 with inhibitors added simultaneously to the medium at 1-20 fold excess. IAPP and inhibitors are not premixed but added separately to the cell culture medium to maximize the similarities to conditions *in vivo*.

10 Cell viability is quantified using the AlamarBlue assay (Biosource International) that provides an easily detectable fluorescent output that does not interfere with the amyloid-cell interactions. Toxicity is assayed both over the short (sampling every 2 hrs over an 8-10 hr period) and long term (every

12 hrs for a period of 6-7days). Data from these investigations will indicate if

15 the inhibitors are able to regulate this more physiological action of IAPP.

**4. Inhibition of Biosynthetic IAPP Fibrils:** Previous studies have demonstrated that islet cultured from IAPP transgenic mice stimulated with glucose will generate extracellular amyloid fibrils. This represents a close

20 approximation to an *in vivo* setting and the inhibitors will be examined for their ability to prevent formation of this biosynthetic IAPP amyloid.

Islet culture from wild-type mice expressing the non-amyloidogenic peptide and transgenic mice over-expressing human IAPP are isolated using established protocols. [Tsujimura, T.; Kuroda, Y.; Tatsuya Kin, T.; Avila, J.G.;

25 Rajotte, R.V.; Korbutt, G., S.; Ryan, E.A.; Shapiro, A.M.; and Lakey, J.R.T. (2002) *Transplantation* 74, 1687-1691] Islets are co-cultured with the most active inhibitors as identified by the CD/EM/toxicity assays (initially at 20-fold excess to obtain maximal effect). Following treatment, cells can be fixed, embedded and sectioned for immunohistochemistry. IAPP amyloid

30 surrounding islets and within intercellular spaces are visualized using Thioflavin S (ThS) which is a fluorescent dye that specifically detects amyloid deposits. IAPP fibrils in multiple sections can be quantified using image

analysis protocols as described previously for the immunization treatments of Alzheimer amyloid transgenic mice.

**EXAMPLE 1 - Minimal Inhibitory Peptide Sequence**

5 Hexapeptides are considered too large to be effective inhibitors under physiological conditions and therefore a smaller active subunit contained within the active hexapeptides ANFLVH (SEQ.ID. NO 11) is useful. To accomplish this goal, systematic truncation of the individual hexapeptides was performed. The complete series of truncated ANFLVH (13-18) (Figure 3) has  
10 been investigated using the *in vitro* assays as described above(CD, EM and cell toxicity) over a typical dosing range (molar ratio from 1:1-1:20). The results from this identified the more active domain(s) within the larger hexapeptide. Three sub-fragments – ANFLV (SEQ. ID. NO. 22), ANFL (SEQ. ID. NO. 23) and ANF (SEQ. ID. NO. 24) – were synthesized and examined in  
15 the RIN-cell toxicity assay using exogenous IAPP (see Figure 6). This initial study has demonstrated that truncated peptides can exhibit activity comparable to the longer precursors. Each of these peptides showed activity, the tripeptide showing greater activity than the tetra- or penta- peptide.

20 **EXAMPLE 2 - Residue Specificity and Optimization**

Not wishing to be bound to any particular theory, the proposed mechanism of action for the peptide fragments is that binding to IAPP disrupts the normal packing of the polypeptide backbone and/or side chain interactions within the fibrils. However, the exact structural basis for these interactions is  
25 not known and it is conceivable that slight modifications, for example, in the inhibitory peptides may improve binding and/or be more effective at disrupting the amyloid polymer packing. To address this possibility, a combinatorial approach was examined by substitution of each residue within the active sequence. For instance, all naturally-occurring amino acids can be used to  
30 generate a peptide library in an effort to optimize the activity of the peptide inhibitors.

Based upon the results from the truncation study, the most active peptides (e.g., trimers and/or tetramers) can be systematically substituted at each residue for a different amino acid. For example, in the case of the ANF peptide (residues 13-15) substitutions can be made at: ANX (SEQ. ID. NO. 5 28), AXN (SEQ. ID. NO. 29), and XNF (SEQ. ID. NO. 30), with X corresponding to: Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asp, Asn, Glu, Gln, Arg, Lys, His, Phe, Tyr, Trp and Pro. This will generate 57 different derivatives that can be examined using an in vitro assay system, such as described below (CD, EM and cell toxicity). This will allow optimization of inhibitor 10 activity and also provide valuable structural information regarding their potential mechanism of action.

To illustrate, the tripeptide ANF was substituted with G at each of the amino acid positions to generate the tripeptides GNF, AGF, and ANG.

Circular dichroism (CD) data for the truncated tripeptide - ANF - 15 indicating the transition from random coil to beta-sheet is shown in Figures 4 A-H. Figures 4A and 4B show that ANF inhibited this conformational change when combined with IAPP at a molar ratio of 1:10. Modifications of this initial inhibitory sequence to generate the peptides - GNF (SEQ. ID. NO. 25) and AGF (SEQ. ID. NO. 26) - resulted in similar but more potent activities at 20 significantly lower molar ratios. (Figures 4C and 4D for GNF (SEQ. ID. NO. 25) and Figures 4E and 4F for AGF (SEQ. ID. NO. 26)). Data for truncated peptide NFL (SEQ. ID. NO. 33) are also shown in Figures 4G and 4H.

Figures 5 A – F are electron micrographs from negatively stained 25 preparations of the full length IAPP (5A) showing the dense network of amyloid fibrils. Figure 6B illustrates that the inhibitory peptide, ANF, reduces the relative levels of fibrils and altered the morphology of the aggregates which were formed. Examination of the substituted peptides (GNF (Figure 5C, (SEQ. ID. NO. 25)), AGF (Figure 5D, (SEQ. ID. NO. 26)) indicated a significantly reduced density of fibrils which is consistent with the CD data 30 (Figure 4). Results for active peptide NFL (SEQ. ID. NO. 33) are shown in Figure 5F). Amorphous aggregates were observed in some instances but virtually no recognizable amyloid-like fibrils were observed. The additional

ANG peptide (Figure 5E, (SEQ. ID. NO. 27)) appeared to have a lower activity based upon the presence of multiple aggregate forms that may explain the lower activity in the toxicity assay (Figure 7).

Figures 6 and 7 illustrate the toxicity data results for the peptides 5 tested. Figure 7 illustrates the results for the optimized tripeptides. The results are consistent with what would be predictable from the CD and EM studies. GNF (SEQ. ID. NO. 25) and AGF (SEQ. ID. NO. 26) appeared to have more activity than ANG (SEQ. ID. NO. 27) or ANF (SEQ. ID. NO. 24).

10 **EXAMPLE 3 - Small Molecule Analogues of Peptide Inhibitors.**

Due to low bioavailability and peripheral degradation, peptide treatments present a number of problems for drug development. Translating peptides into a small molecule mimetic is often difficult due to the typical size of the active sequences (hexamers and larger). However, the observation 15 that tripeptides display significant activity brings them into a molecular weight range more tractable in terms of predicting the active structure and equivalent small molecule analogues.

The approaches would involve molecular modeling and energy minimization to obtain a likely structure of the peptide fragment. Using this as 20 a template, it would be possible to chemically synthesize a small organic molecule that resembled this structure. Any inhibitory molecules would then be optimized using standard structure-activity-relationship approaches based upon the original organic compounds.

Alternatively, a direct structural approach could be taken where, for 25 example, the ANF peptide was combined with full-length IAPP. The ANF would by virtue of its inhibitory properties maintain IAPP in a soluble state that could be amenable to NMR analysis. This strategy could generate a 3D molecular structure of the bound inhibitor to reveal the active conformation. Information of this sort could then be used to design more effective mimetics 30 of the ANF peptide.

**EXAMPLE 4 - Examining the Effects of Amyloid Inhibitors on Islet**

### Survival

Culturing human islets is often problematic and yields, for example, from donors used for transplant purposes are often highly variable. It has been proposed that this could be due to intrinsic pancreatic proteases such as

5 trypsin and/or a sensitivity to oxidative stress. In contrast, mouse islets appear to be more robust and are stable in culture for several days. Although postmortem effects may come into play, one possible explanation is that the propensity of human IAPP to form amyloid may contribute to the observed cell death. The inability of murine IAPP to undergo this transformation and its  
10 more soluble nature may provide a protective effect leading to more viable cells. This is supported by the observation that amyloid was formed rapidly in transplanted islet expressing human IAPP leading and promoted cell death. Amyloid-related toxicity may therefore have implications for transplantation therapies which are currently being used to treat type-2 diabetes.

15 To test this hypothesis, islets were isolated from different transgenic animals and their survivability in culture examined. Transgenic mice were generated using standard methods. This involved a full-length human IAPP (including the pro-sequence) with expression driven by the rat insulin promoter. (Figure 8) The transgenic mice also expressed mouse IAPP which  
20 may influence the process of amyloidogenesis. To avoid this complication the animals were also crossed onto an IAPP knockout line to obtain a humanized version of the transgene, at least as far as IAPP is concerned. The method used herein is similar to that described in Verchere, C.B., D'alessio, D.A., Palmiter, R.D., Weir, G.C., Bonner-Weir, S., Baskin, D.G. and Kahn, S.E.  
25 (1996) Proc. Natl. Acad. Sci. USA 93: 3492-3496), with some modifications.

Initial studies using transgenic islets have revealed a high level of IAPP secretion as measured by a commercial ELISA (Linco Research). (Figure 9) Interestingly, islets extracted from animals fed on a high fat diet appeared to have a low level of secretions possibly due to  $\beta$  cell exhaustion. ). (Figure 10).  
30 The diets used in these studies were similar to that described in Verchere, C.B., D'alessio, D.A., Palmiter, R.D., Weir, G.C., Bonner-Weir, S., Baskin, D.G. and Kahn, S.E. (1996) Proc. Natl. Acad. Sci. USA 93: 3492-3496.

For the proposed investigation, islets were extracted from: (1) wild-type control mice; (2) transgenics over-expressing human IAPP on a normal mouse background; and (3) transgenics on an IAPP knockout background. There should be a defined gradient in viability for these cultures with the 5 lowest survival being seen for islets on the IAPP ablated background. This represents the closest approximation of a human culture and should have the greatest amyloid load. Intermediate viability should be observed for islets over-expressing human IAPP as attenuation of the amyloid pathway by the endogenous murine protein is anticipated.

10 Another complicating factor is that islets often exhibit necrosis within their core most likely due to poor perfusion of these cells. To avoid this problem, viability analysis will be performed on dissociated islets. This is accomplished by trypsinization and passing the cells through a 60  $\mu$ m spectra mesh (Spectrum Labs Inc.). Dissociated islets can be cultured in high (16.7 15 mmol/l) glucose which is sufficient to stimulate IAPP fibril formation and compared to cells exposed to low (4.2 mmol/l) glucose. As with the toxicity assays, cell viability can be determined over the course of 3-4 days using the AlamarBlue assay. Results of this study can be seen in Figure 11. To confirm that cell death/survival correlates with amyloid load, fixed but 20 unpermeabilized cells can be stained for human IAPP using specific antibody and examined by immunofluorescence. This allows estimation of the amount of extracellular amyloid that has been deposited surrounding cells and in association with their plasma membrane.

The polyclonal antibody used in the present invention was generated 25 using a synthetic peptide antigen corresponding to residues 8-37 of the human IAPP. This was used to immunize rabbits and antibodies were produced using a standard protocol.

Electron microscopy images of cultured islets isolated from transgenic mice expressing the human IAPP protein were taken. (Figure 12) IAPP 30 amyloid fibrils are visible in the interstitial spaces between the cells (center of the image) and radiating out of the plasma membranes. These are typical amyloid-like fibrils that have been observed in similar islet cultures derived

from transgenic mice. [de Konig, E.J.P., Morris, E.R., Hofhuis, F.M.A., Posthuma, G., Hoppener, J.W.M., Morris, J.F., Cael, P.J.A., Clark, A. and Verbeek, J.S., (1994) Proc. Natl. Acad. Sci. USA 91: 8467-8471; de Koning, E.J.P., Bodkin, N.L., Hansen, B.C. and Clark, A. (1993) Diabetologia 36:378-5 384]

This investigation can be extended to examine if the most active IAPP inhibitors (derived from peptide studies and Innodia small molecules) are able to increase islet survival. The outcomes from the investigation as a whole can: (1) provide additional support for a significant role of IAPP amyloid in 10 islet cell death; (2) further validate the effectiveness of the inhibitors which have been developed; and (3) provide a new and potentially important tool for the treatment of human islets that can be used to isolate cleaner and more 20 viable preparations from donor for transplantation therapies which are currently being used for type-2 diabetes.

15

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the 20 invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

25

INDEX TO SEQUENCE IDENTIFICATION NUMBERS

SEQ. ID. NO.	DESCRIPTION
1	Human IAPP 37 aa
2	Mouse IAPP – 37 aa
3	A.A. 8 –18 of hIAPP
4	A.A. 20-29 of hIAPP
5	A.A. 31-37 of hIAPP
6	ATQRLA
7	TQRLAN
8	QRLANF
9	RLANFL
10	LANFLV
11	ANFLVH
12	NFLVHS
13	FLVHSS
14	SSNNFG
15	SNNFGA
16	NNFGAI
17	NFGAIL
18	FGAILS
19	GAILSS
20	AILSST
21	ILSSTN
22	ANFLV
23	ANFL
24	ANF
25	GNF
26	AGF
27	ANG

<b>SEQ. ID. NO.</b>	<b>DESCRIPTION</b>
28	ANX, where X is any A.A.
29	AXF, where X is any A.A.
30	XNF, where X is any A.A.
31	NFLVH
32	FLVH
33	NFL
34	LVH
35	FLV
36	GSNKGAIIGL ( $\beta$ -IAPP, 25-34)
37	HVAAGAVVGG (PrP, 110-119)
38	ATQRLANFLVHSS
39	SSNNFGAILSSTN

**WE CLAIM:**

1. An antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection, comprising a peptide selected from the group consisting of penta-, tetra, and tri- peptides of truncated ANFLVH (SEQ ID NO. 11), an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
- 5 2. The antifibrillogenic agent of claim 1, wherein said peptide is ANFLV (SEQ ID NO. 22), ANF (SEQ ID NO. 24), or NFL (SEQ. ID. NO. 33), an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
- 10 3. An antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection of claim 1, wherein the agent comprises a tripeptide selected from the group consisting of ANF (SEQ ID NO. 24), ANX (SEQ. ID. NO. 28); AXF (SEQ. ID. NO. 29); or XNF (SEQ. ID. NO. 30), where X is any amino acid except cysteine, an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
- 15 4. The antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection of claim 3, wherein the tripeptide is selected from the group consisting of ANF (SEQ ID NO. 24), GNF (SEQ. ID. NO. 25), AGF (SEQ. ID. NO. 26), , an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
- 20 5. The antifibrillogenic agent of any one of claims 1 - 4, wherein said agent is an all-[D] isomer of said peptide.
- 25 6. The antifibrillogenic agent of any one of claims 1 to 4, wherein said agent is an all-[L] isomer of said peptide.
7. The antifibrillogenic agent of any one of claims 1 to 4, wherein said agent contains a mixture of [L] and [D] isomers of said peptide.

8. A peptide for inhibiting amyloidosis and/or cytoprotection, said peptide selected from the group consisting of penta-, tetra, and tri- peptides of truncated ANFLVH (SEQ ID NO. 11), an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
- 5 9. The peptide of claim 8, wherein said peptide is ANFLV (SEQ ID NO. 22), or ANF (SEQ ID NO. 24).
10. A peptide of claim 8 for inhibiting amyloidosis and/or cytoprotection, said peptide comprising a sequence selected from the group of tripeptides consisting of ANF (SEQ ID NO. 24), ANX (SEQ. ID. NO. 28); AXF (SEQ. ID. NO. 29); or XNF (SEQ. ID. NO. 30), where X is any amino acid except cysteine, an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
11. The peptide of claim 10, wherein the peptide is selected from the group consisting of: ANF (SEQ ID NO. 24), GNF (SEQ. ID. NO. 25), AGF (SEQ. ID. NO. 26), an isomer thereof, a retro or a retro-inverso isomer thereof, a peptidomimetic thereof or a salt thereof.
12. The tripeptide of claim 11, wherein said sequence is ANF (SEQ ID NO. 24).
13. The tripeptide of claim 10, wherein said amyloidosis is IAPP-related.
- 20 14. The tripeptide of claim 10, wherein said amyloidosis is type 1 or type 2 diabetes.
15. A composition for inhibiting amyloidosis and/or for cytoprotection, comprising a therapeutically effective amount of the peptide of claim 8 in association with a pharmaceutically acceptable carrier.
- 25 16. A composition for inhibiting amyloidosis and/or for cytoprotection, comprising a therapeutically effective amount of the peptide of any one of claims 9 - 11 in association with a pharmaceutically acceptable carrier.

17. A compound for inhibiting amyloidosis and/or for cytoprotection, where said compound binds with the peptide of claim 8 or 16.
18. The compound of claim 17, wherein said compound is an enzyme that binds to or controls the expression of the peptide.
- 5 19. The compound of claim 18, wherein said compound is an antibody that binds to the peptide.
20. The compound of claim 19, wherein said antibody specifically binds to the peptide.
- 10 21. The compound of claim 20, wherein said antibody is a monoclonal antibody.
22. The compound of claim 17 wherein said compound is a salt.
23. A labeled conjugate for *in vivo* imaging of amyloid deposits, comprising a conjugate of formula I:

$$A_t - (A_{\text{link}}_z) - A_{\text{lab}} \quad (\text{I})$$

15 where  $z$  is 0 or 1;  $A_t$  is the antifibrillogenic agent of any one of claims 1 to 7;  $A_{\text{link}}$  is a linker moiety; and  $A_{\text{lab}}$  is a labeling moiety that allows for said *in vivo* imaging.

24. The labeled conjugate of claim 23, wherein said agent is an all-[D] isomer peptide.
- 20 25. The labeled conjugate of claim 23, wherein said agent is an all-[L] isomer peptide.
26. The labeled conjugate of claim 23, wherein  $A_{\text{lab}}$  is a radiolabeling moiety.
27. The labeled conjugate of claim 26, wherein  $A_{\text{lab}}$  is selected from the group consisting of  $^{99m}\text{Tc}$ ,  $^{99}\text{Tc}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ,  $^{97}\text{Ru}$ ,  $^{119}\text{Pd}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,

$^{111}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{153}\text{Gd}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{166}\text{Ho}$ ,  $^{198}\text{Au}$ ,  $^{90}\text{Sr}$ ,  $^{89}\text{Sr}$ ,  $^{115}\text{Rh}$ ,  $^{201}\text{TI}$ ,  $^{51}\text{Cr}$ ,  $^{67}\text{Ga}$ ,  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$  and  $^{18}\text{F}$ .

28. The labeled conjugate of claim 23, wherein said amyloid deposits comprise IAPP amyloid.
- 5 29. The labeled conjugate of claim 23, wherein said amyloid deposits are associated with type 1 or type 2 diabetes.
30. A composition for *in vivo* imaging of amyloid deposits, comprising a therapeutically effective amount of the labeled conjugate of claim 23, and a pharmaceutically acceptable carrier.
- 10 31. A composition for the treatment of amyloidosis disorders in a patient, comprising a therapeutically effective amount of the labeled conjugate of claim 23, and a pharmaceutically acceptable carrier.
32. A method for the treatment of amyloidosis disorders in a patient, comprising administering to said patient a therapeutically effective amount of the antifibrillogenic agent of any one of claims 1 to 7.
- 15 33. The method of claim 32, wherein said amyloidosis disorder is IAPP-related.
34. The method of claim 33, wherein said amyloidosis disorder is type 1 or type 2 diabetes.
- 20 35. The method of claim 34, wherein said antifibrillogenic agent is administered in conjunction with another agent selected from the group consisting of insulin, sulfonylurea and glucose sensitizers.
36. A method for the treatment of amyloidosis disorders in a patient, comprising administering to said patient a therapeutically effective amount of the composition of claim 31.

37. The method of claim 36, wherein said amyloidosis disorder is IAPP-related.
38. The method of claim 37, wherein said amyloidosis disorder is type 1 or type 2 diabetes.
- 5 39. The method of claim 37, wherein said composition is administered in conjunction with another agent selected from the group consisting of insulin, sulfonylurea and glucose sensitizers.
- 10 40. A process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting cells *in vitro* with the antifibrillogenic agent of any one of claims 1 to 7 for inhibiting amyloid deposit formation.
- 15 41. The process of claim 40, wherein said antifibrillogenic agent causes breakdown of amyloid deposits, the deposits having been formed by said cells prior to said contact.
42. The process of claim 40, wherein said cells are cultured in the presence of said antifibrillogenic agent.
43. The process of claim 40, wherein said amyloid deposits comprise IAPP amyloid.
- 20 44. The process of claim 40, wherein said amyloid deposits are associated with type 1 or type 2 diabetes.
45. The process of claim 40, wherein said cells, prior to treatment, form amyloid deposits.
46. Cells suitable for transplantation into a mammal, which have been prepared by the process of claim 40.

47. A method for treating a Type I diabetes patient after transplantation, said method comprising the step of administering *in vivo* to said patient the antifibrillogenic agent of any one of claims 1 to 7 for inhibiting, preventing and/or reducing amyloid deposit formation and amyloidosis.
- 5 48. The method of claim 47, wherein said amyloid deposit formation and/or amyloidosis is IAPP-related.
49. The method of claim 47, wherein said composition is administered in conjunction with another agent selected from the group consisting of insulin, sulfonylurea and glucose sensitizers.
- 10 50. A method for inhibiting amyloidosis and/or for cytoprotection, comprising administering to a subject a therapeutically effective amount of the antifibrillogenic agent of any one of claims 1 to 7, wherein said antifibrillogenic agent prevents or reduces amyloid deposition.
- 15 51. The method of claim 50, wherein said antifibrillogenic agent is administered by cell therapy or gene therapy, wherein cells have been modified to produce and secrete the antifibrillogenic agent.
52. The method of claim 51, wherein said cells have been modified *ex vivo*.
53. The method of claim 51, wherein said cells have been modified *in vivo*.
- 20 54. The method of claim 50, wherein said amyloidosis or amyloid deposition is IAPP-related.
55. The method of claim 50, wherein said amyloidosis or amyloid deposition is type 1 or type 2 diabetes.
- 25 56. The method of claim 50, wherein said composition is administered in conjunction with another agent selected from the group consisting of insulin, sulfonylurea and glucose sensitizers.

57. A method for identifying an optimized peptide for inhibition of amyloidogenesis comprising,

5 (a) choosing an original peptide ANF (SEQ ID NO. 11),

10 (b) systematically substituting at each residue a different amino acid,

15 (c) testing the ability for each derivative to inhibit amyloid fibril formation,

(d) comparing the inhibition of each derivative with the inhibition of the original peptide,

wherein an increase in inhibition of the derivative over the original peptide indicates an optimized peptide.

58. The method of claim 57, wherein the different amino acid is chosen from the group consisting of Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asp, Asn, Glu, Gln, Arg, Lys, His, Phe, Tyr, Trp and Pro.

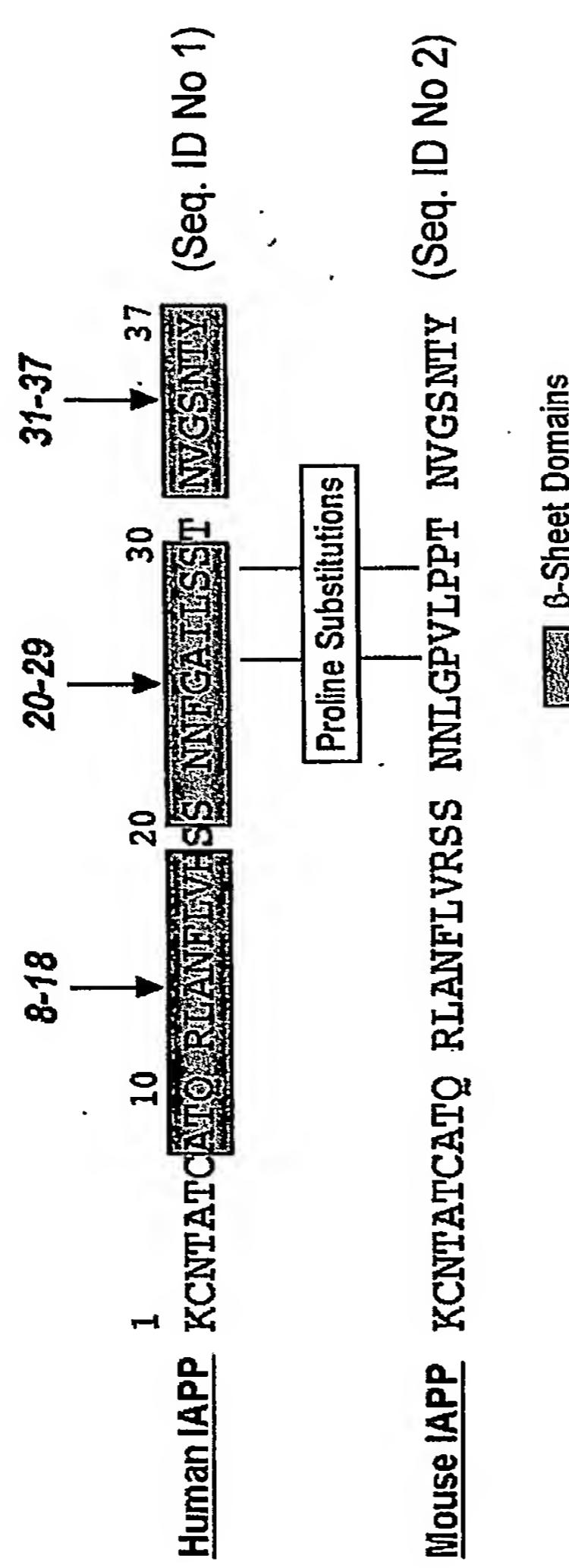
59. The method of claim 57 wherein the testing for inhibition consists of at least one of the following *in vitro* assay systems: CD, EM and cell toxicity.

15

60. The optimized peptide identified using the method of claim 57.

**ABSTRACT OF THE DISCLOSURE**

New antifibrillogenic agents and compositions containing same, methods for optimizing antifibrillogenic agents, methods of using the antifibrillogenic agents and compositions for inhibiting amyloid fibril formation, 5 and effective therapeutics for preventing or delaying the progression of, e.g., Alzheimer's disease and diabetes.



19

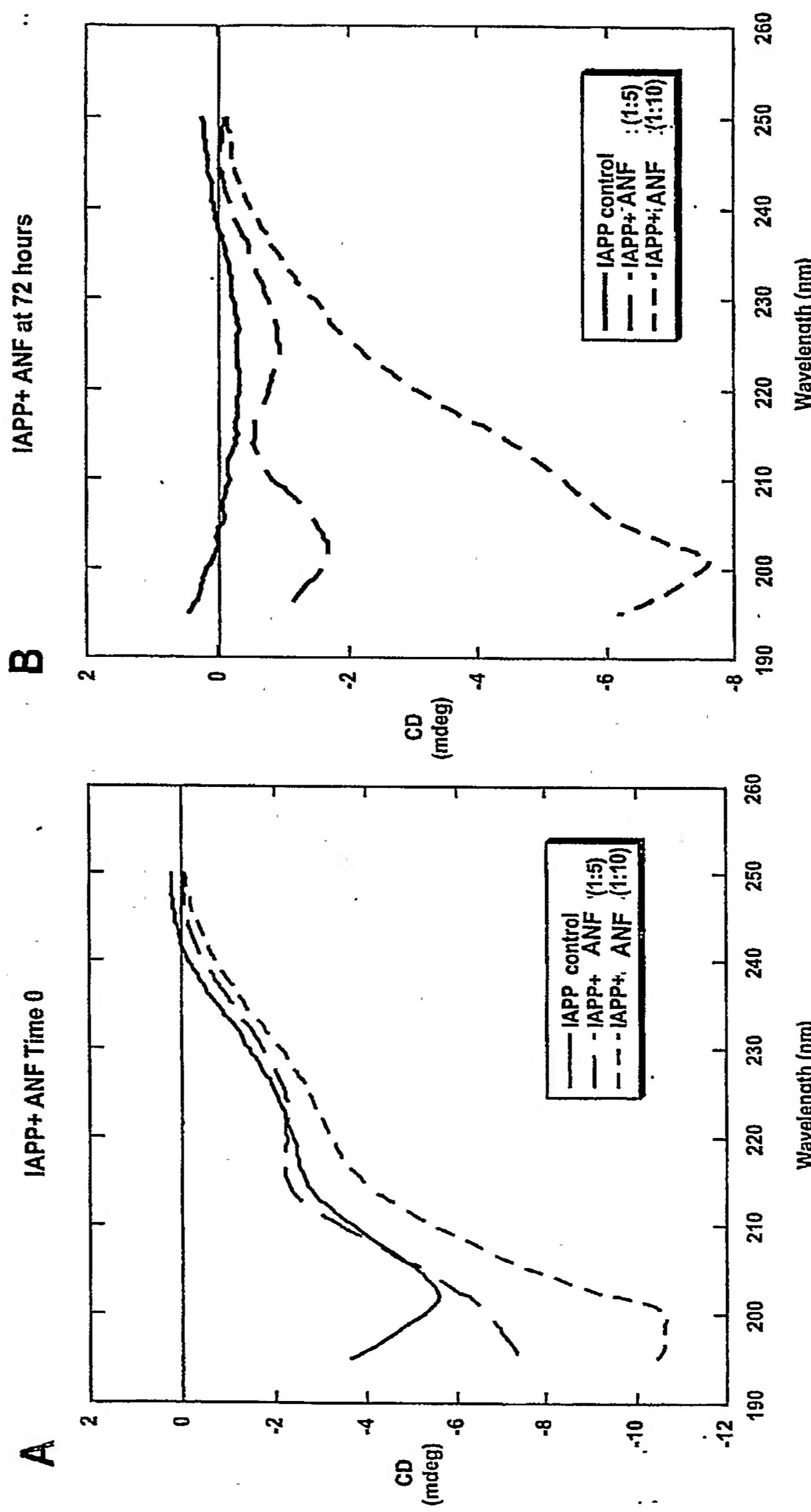
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KCNTATCATQRILANFLVHSSNNFGAILSSTNVGSNTY (Seq. ID No. 1)				
Seq. ID No. 6) ATQRILA		SSNNFG	(Seq. ID No. 14)	
(Seq. ID No. 7) TQRILAN		<u>SNNFGA</u>	(Seq. ID No. 15)	
(Seq. ID No. 8) QRILANF		NNFGAI	(Seq. ID No. 16)	
(Seq. ID No. 9) RLANFL		NFGAIL	(Seq. ID No. 17)	
(Seq. ID No. 10) LANFLV		FGAILS	(Seq. ID No. 18)	
(Seq. ID No. 11) <u>ANFLVH</u>		<u>GAILSS</u>	(Seq. ID No. 19)	
(Seq. ID No. 12) <u>NFLVHS</u>		AILSST	(Seq. ID No. 20)	
(Seq. ID No. 13) FLVHSS		ILSSTN	(Seq. ID No. 21)	

**Fig 2**

Optimization of IAPP Peptide Inhibitors

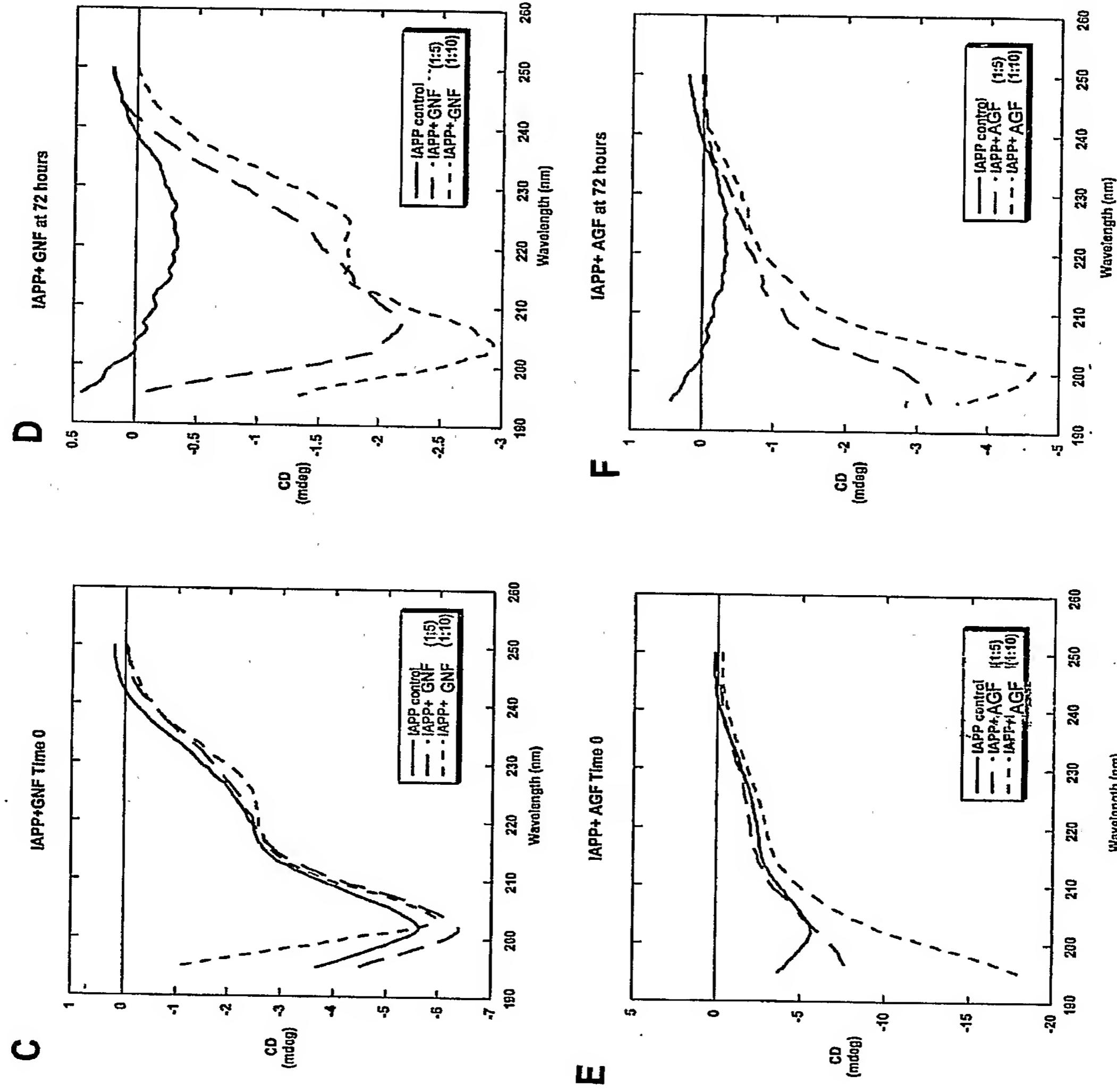
IAPP 13-18	IAPP 14-19
ANFLVH (Seq ID No. 11)	NFLVHS (Seq ID No. 12)
NFLVH (Seq ID No. 31R)	FLVHS
FLVH (Seq. ID No. 32)	LVHS
LVH (Seq ID No. 34)	VHS
FLV (Seq ID No. 35)	LVH (Seq ID No. 34)
NFL (Seq ID No. 33)	FLV (Seq ID No. 35)
ANFLV (Seq ID No. 22)	NFLVH
ANFL (Seq ID No. 23)	NFLV
ANF (Seq ID No. 24)	NFL (Seq ID No. 33)

**Fig 3**



**Fig 4**

Fig 4



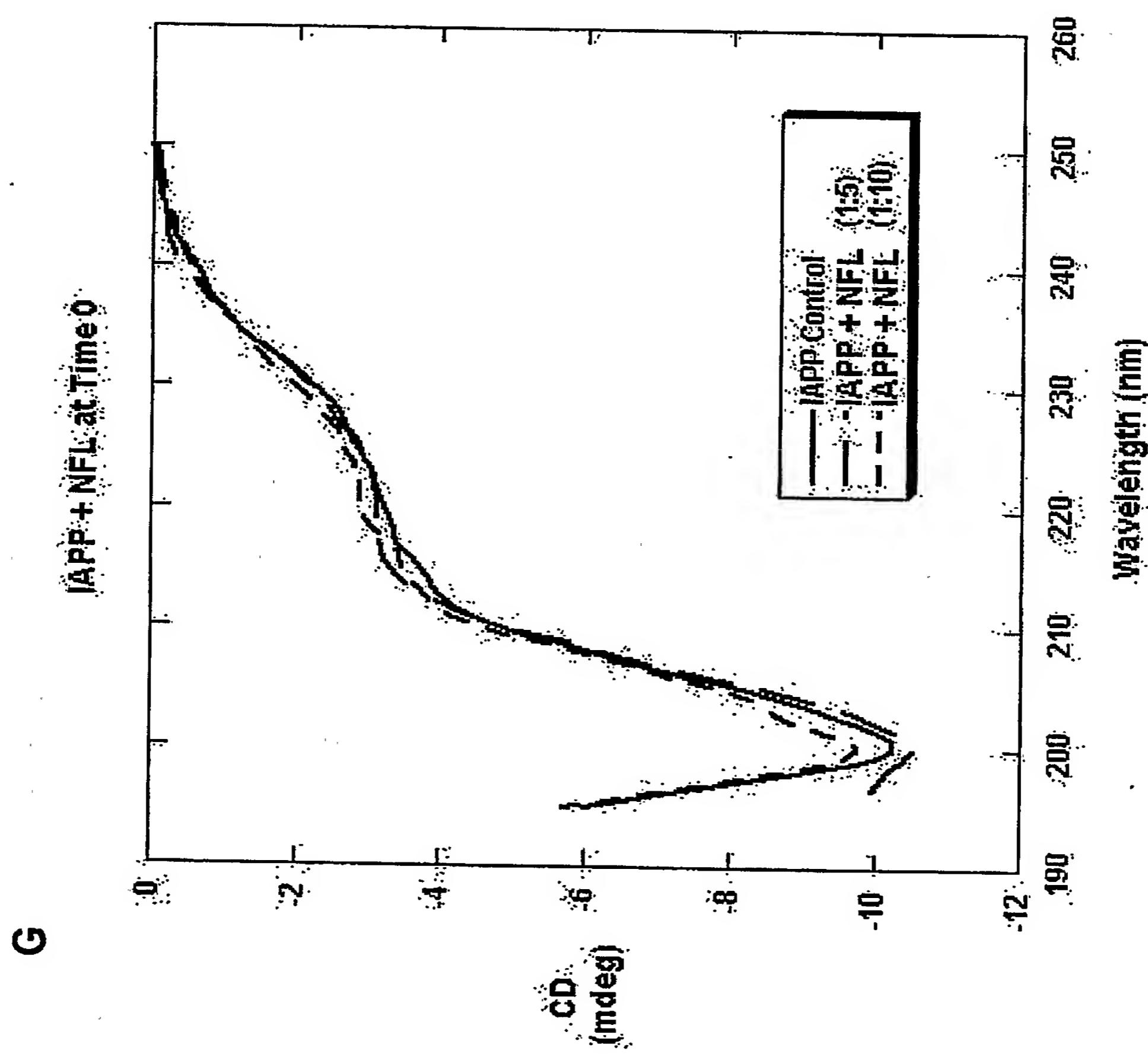


Fig 4

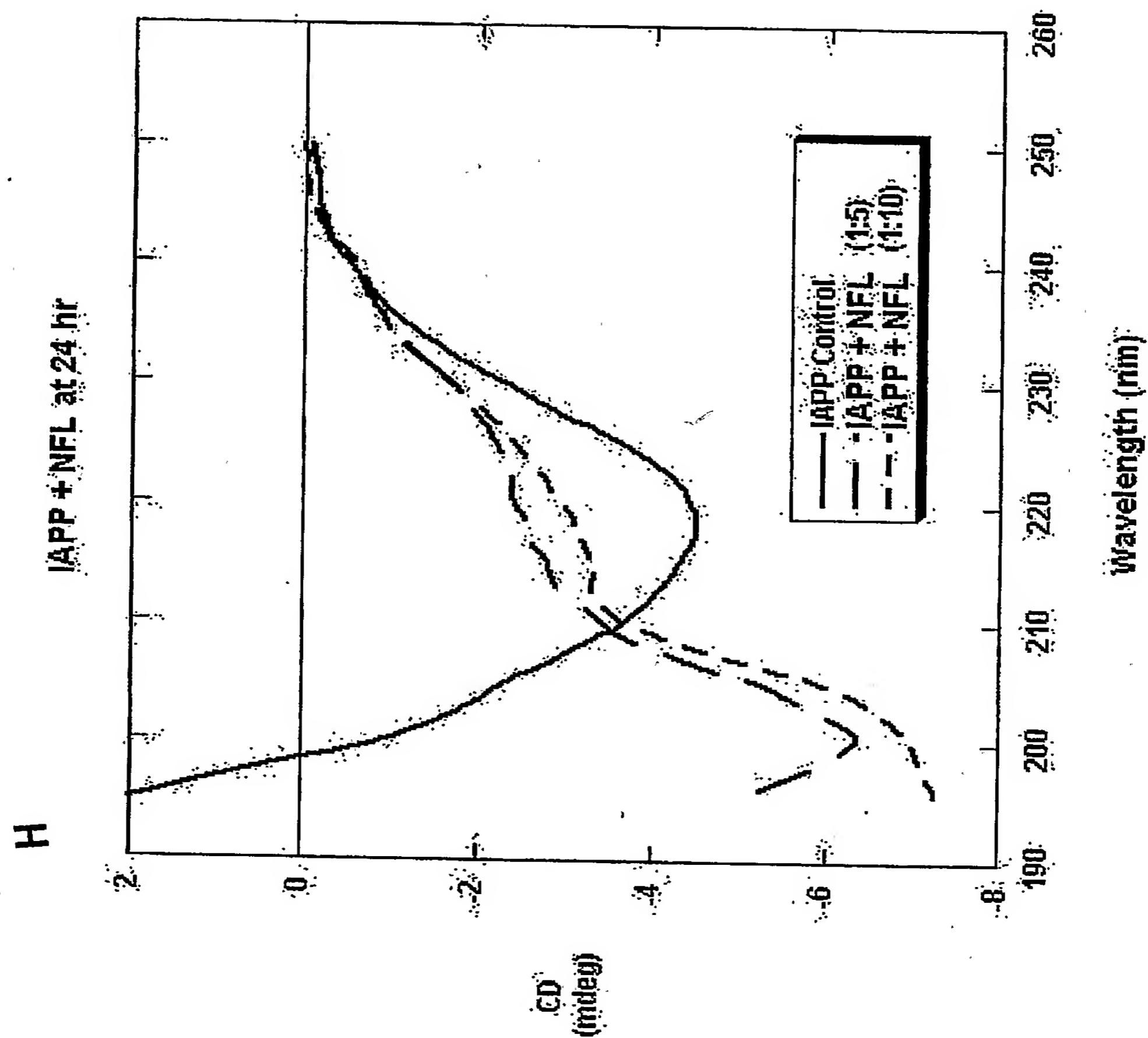


Fig 4

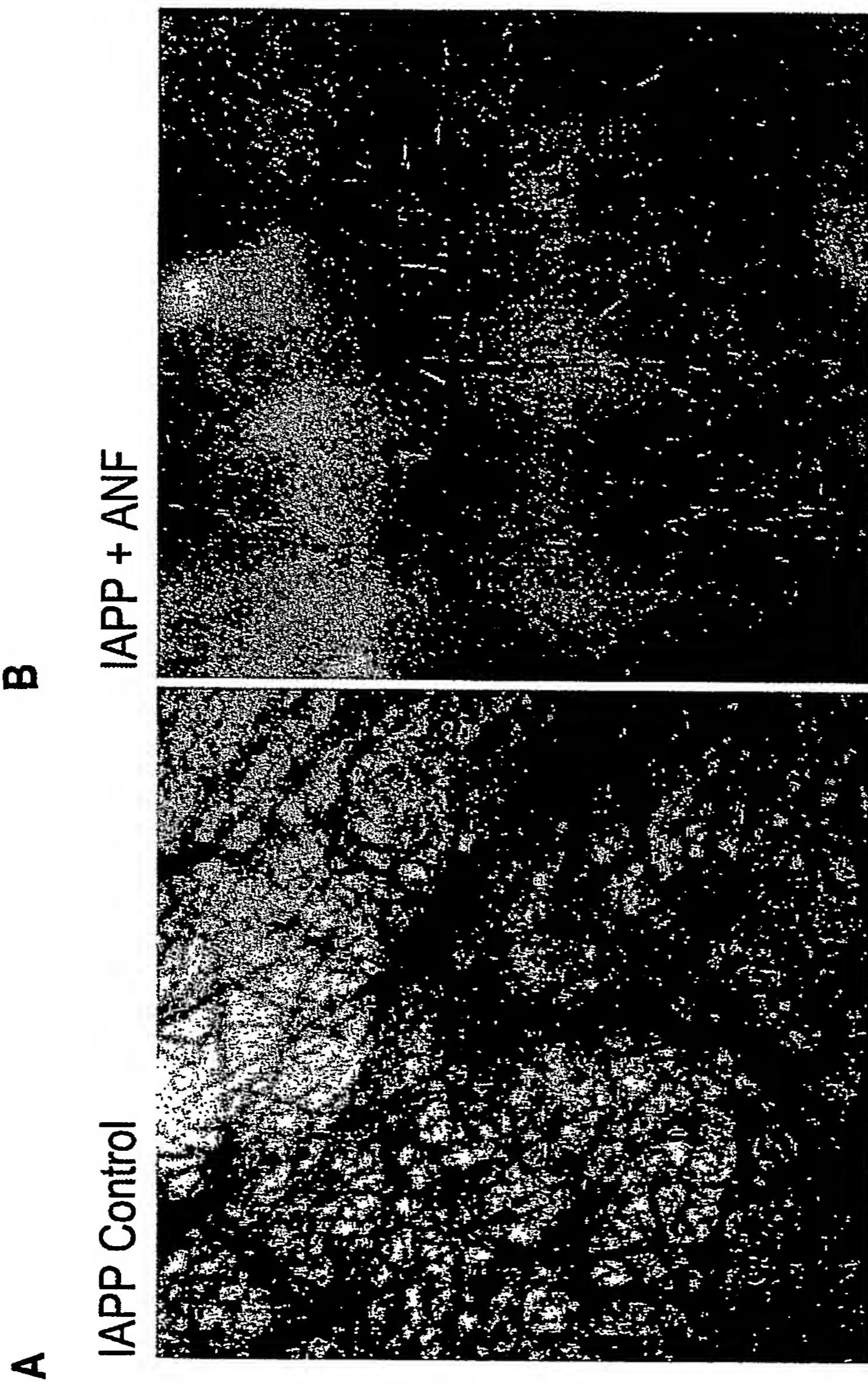


Fig 5

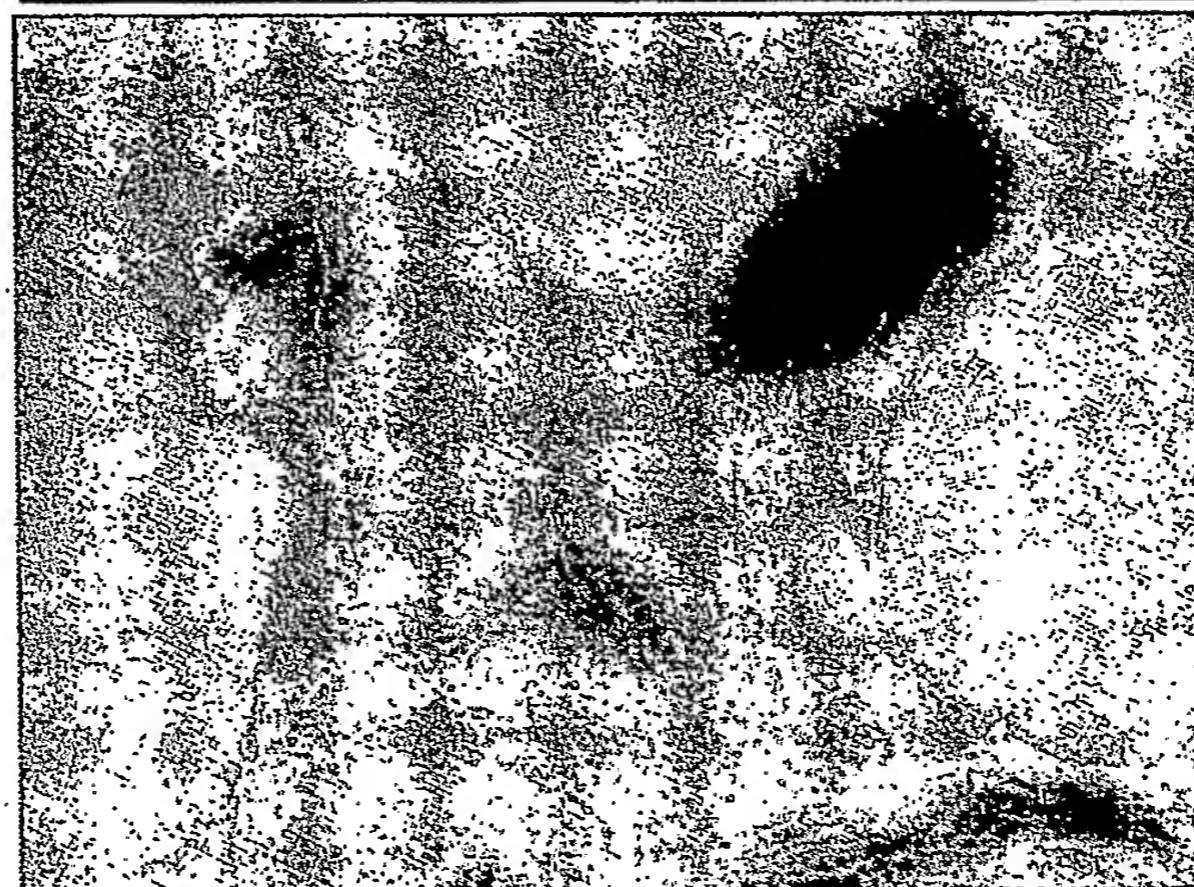
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IAPP + GNF



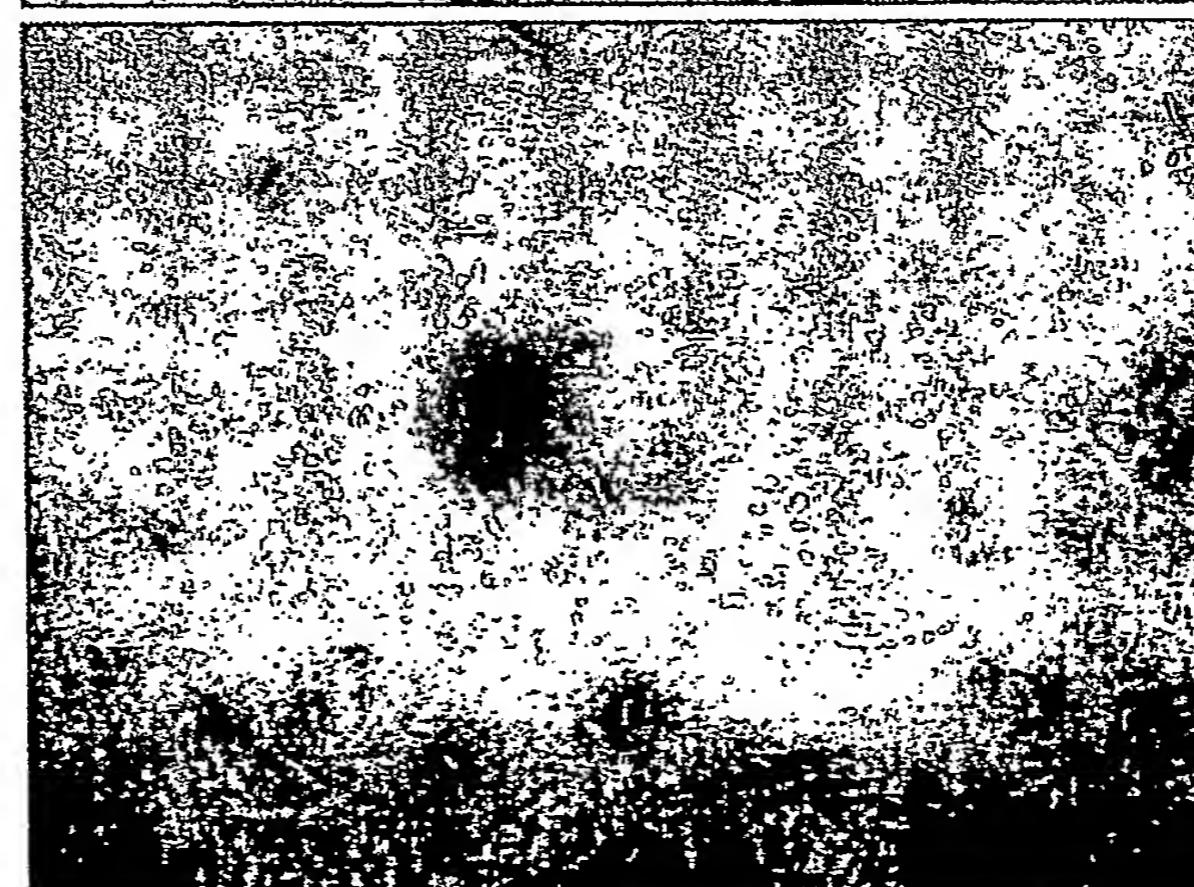
C

IAPP + AGF



D

IAPP + ANG



E

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Fig 5

IAPP + NFL [molar ratio 1:10]



F

**Fig 5**

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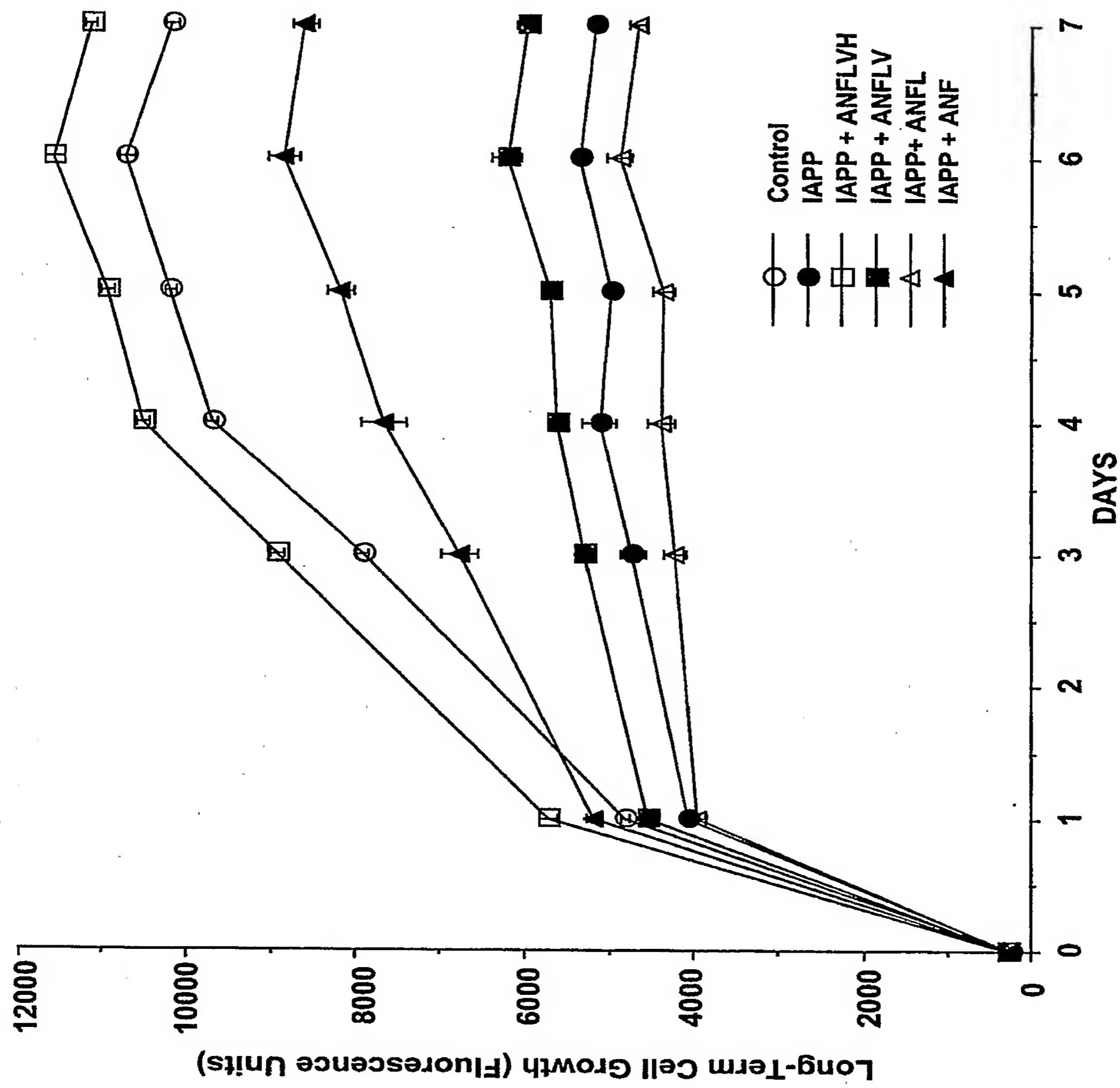


Fig 6

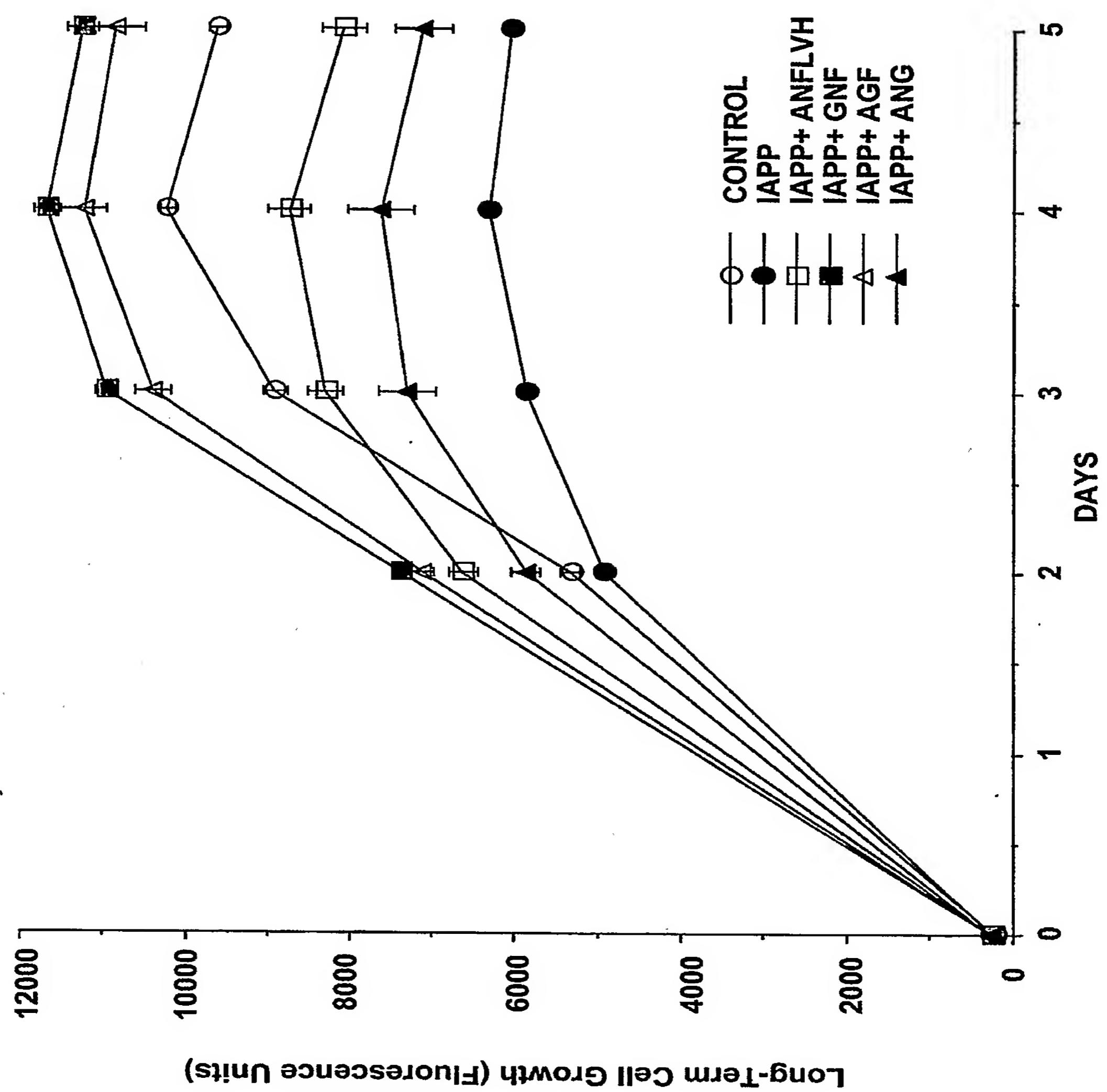


Fig 1

# Human IAPP Transgenic Mice

pNASSMAR.RIP.hIAPP

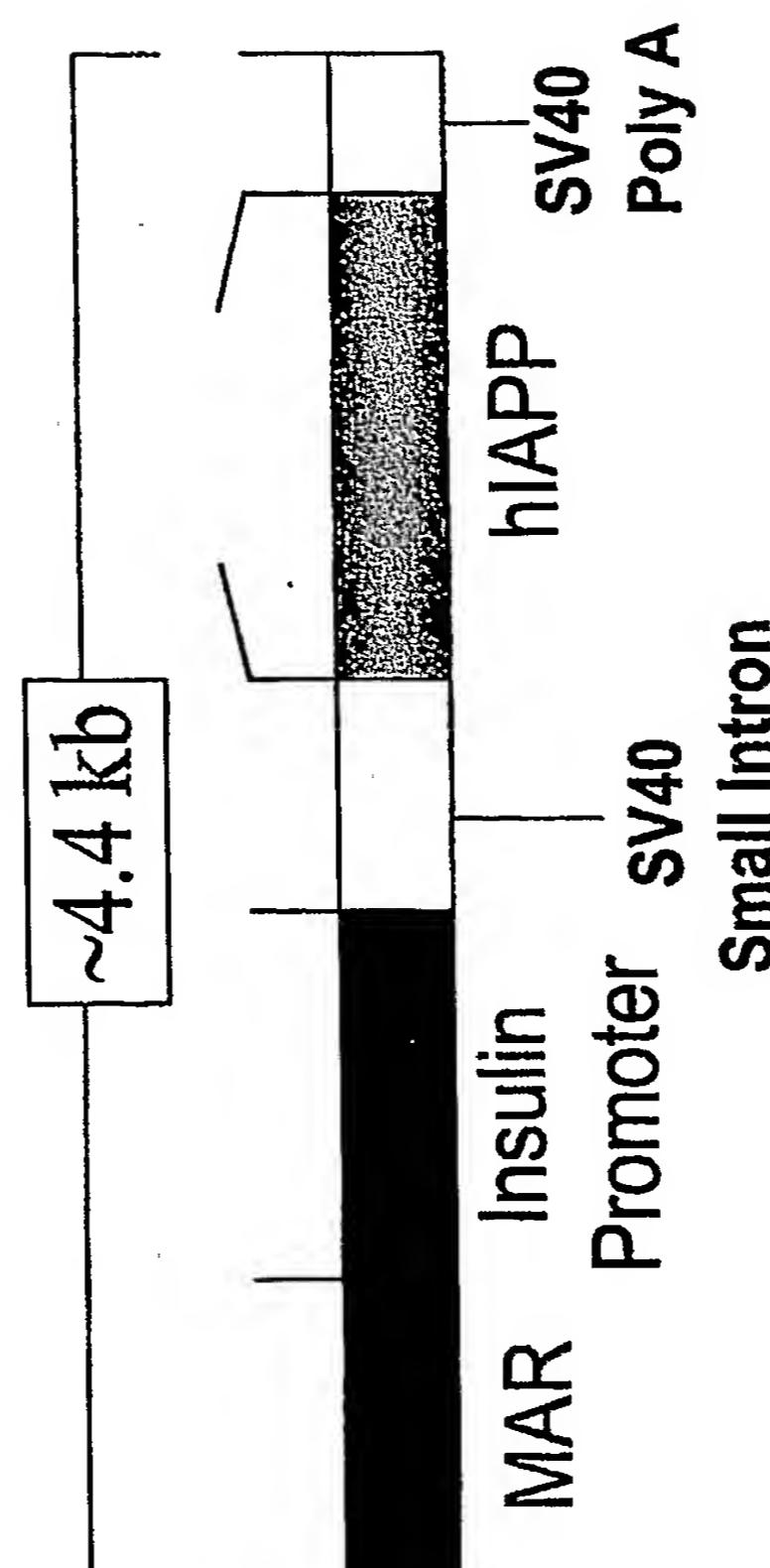
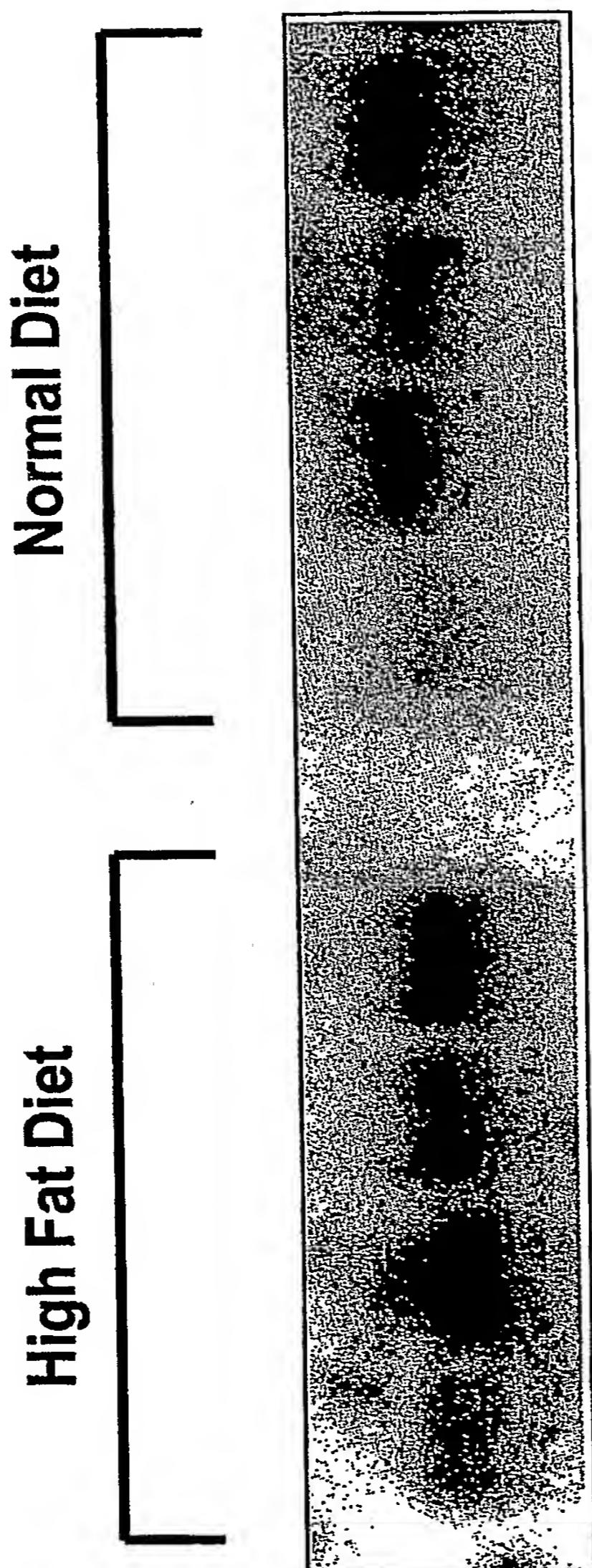


Fig 8

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Wt Tg+ Wt Tg+ Wt Tg+ Wt Tg+

Fig 9

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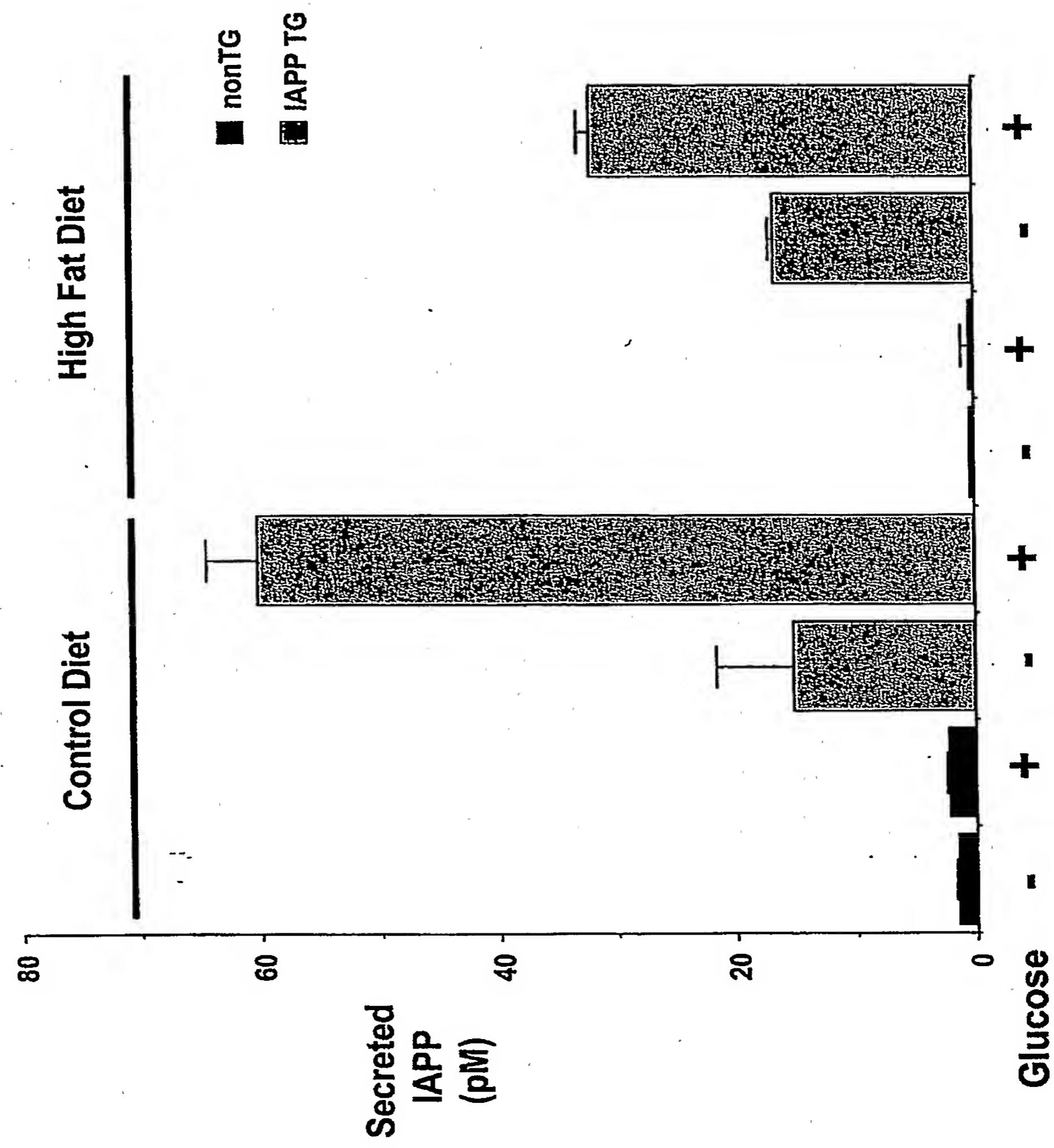


Fig 10

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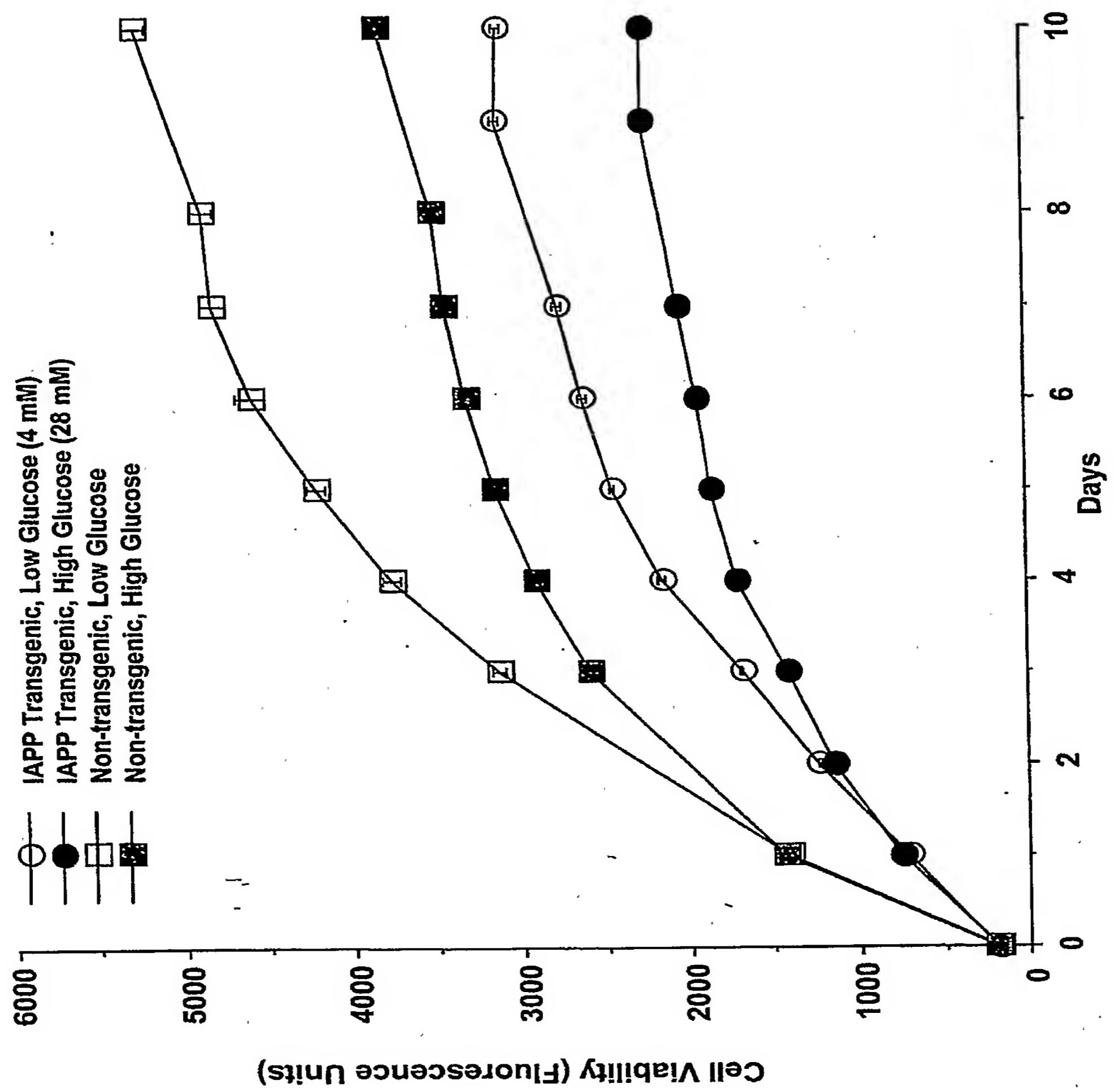


Fig 11

Amyloid Fibrils in Cultured Islets



Fig 12.

B

A

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## Patent Application Data Sheet

### Application Information

Application number::

Application Type:: **Provisional**

Subject Matter:: **Utility**

Suggested

Classification::

Suggested Group Art

Unit::

CD-ROM or CD-R?:: **None**

Number of CD disks::

Number of copies of CDs::

Sequence submission?::

Computer Readable

Form (CRF)?:: **No**

Number of copies of CRF::

Title:: **Inhibitors Of Islet Amyloid Polypeptide (IAPP) Fibril Formation And Uses Thereof**

Attorney Docket Number:: **2223-170**

Request for Early

Publication?:: **No**

Request for Non-Publication?:: **No**

Suggested Drawing Figure::

Total Drawing Sheets:: **17**

Small Entity?:: **Yes**

Latin Name::

Variety denomination

name::

Petition included?:: No

Petition Type::

Licensed US Govt.

Agency::

Contract or Grant

Numbers::

Secrecy Order in

Parent Appl.?:: No

### **Applicant Information**

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Status:: Full Capacity

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**Representative**  
**Customer Number::** 001059

### **Domestic Priority Information**

<b>Application::</b>	<b>Continuity Type::</b>	<b>Parent Application::</b>	<b>Parent Filing Date::</b>
This Application	Continuation of	Enter the parent application number on which priority is claimed	MM/DD/YY
Or enter the appropriate application serial no.		No more than 20 characters	

## Foreign Priority Applications

Country::	Application Number::	Filing Date::	Priority Claimed
			No

## Assignee Information

Assignee name::

Street of mailing address::

City of mailing address::

State or Province of

mailing address::

Country of mailing address::

Postal or Zip Code of

mailing address::